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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C07K 14/435, 14/705, C09K 11/06, G01N 33/53, 33/566

A1

(11) International Publication Number: **WO 99/18124**

(43) International Publication Date: 15 April 1999 (15.04.99)

(21) International Application Number: PCT/US98/21049

(22) International Filing Date: 6 October 1998 (06.10.98)

(30) Priority Data:
60/061,385 7 October 1997 (07.10.97) US

(71) Applicant *(for all designated States except US)*: MERCK & CO., INC. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: CUMMINGS, Richard, T. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). HERMES, Jeffrey, D. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). MOLLER, David, E. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). ZHOU, Gaochao [CN/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).

(74) Common Representative: MERCK & CO., INC.; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).

(81) Designated States: CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) **Title:** ASSAYS FOR NUCLEAR RECEPTOR LIGANDS USING FRET

(57) **Abstract**

Provided is a method of identifying agonists and antagonists of nuclear receptors that comprises measuring agonist–dependent fluorescence resonance energy transfer (FRET) between a fluorescently labeled nuclear receptor or ligand binding domain and fluorescently labeled CREB–binding protein (CBP), p300, other nuclear co–activator, or binding portion thereof. The method is simple, rapid, and inexpensive. Nuclear receptors and nuclear receptor co–activators labeled with fluorescent reagents for use in the above–described method are also provided.

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TITLE OF THE INVENTION

ASSAYS FOR NUCLEAR RECEPTOR LIGANDS USING FRET

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 60/061,385, filed 10/7/97, the contents of which are incorporated herein by reference in their entirety.

STATEMENT REGARDING FEDERALLY-SPONSORED R&D

Not applicable.

REFERENCE TO MICROFICHE APPENDIX

Not applicable.

FIELD OF THE INVENTION

This invention relates to methods of identifying novel agonists and antagonists of nuclear receptors utilizing the agonist-dependent interaction of such receptors with CREB-binding protein (CBP) or other nuclear receptor co-activators in which this interaction is detected by fluorescence resonance energy transfer.

BACKGROUND OF THE INVENTION

Nuclear receptors are a superfamily of ligand-activated transcription factors that bind as homodimers or heterodimers to their cognate DNA elements in gene promoters. The superfamily, with more than 150 members, can be divided into subfamilies (*e.g.* the steroid, retinoid, thyroid hormone, and peroxisome proliferator-activated [PPAR] subfamilies). Each subfamily may consist of several members which are encoded by individual genes (*e.g.* PPARα, PPARγ, and PPARδ). In addition, alternative mRNA splicing can result in more than one isoform of these genes as in the case of specific PPARs (*e.g.* PPARγ1 and PPARγ2). The nuclear receptor superfamily is involved in a wide variety of physiological functions in mammalian cells: *e.g.*, differentiation, proliferation, and metabolic homeostasis. Dysfunction

or altered expression of specific nuclear receptors has been found to be involved in disease pathogenesis.

The PPAR subfamily of nuclear receptors consists of three members: PPARα, PPARγ, and PPARδ. PPARα is highly expressed in liver and kidney. Activation of PPARα by peroxisome proliferators (including hypolipidimic reagents such as fibrates) or medium and long-chain fatty acids is responsible for the induction of acyl-CoA oxidase and hydratase-dehydrogenase (enzymes required for peroxisomal β-oxidation), as well as cytochrome P450 4A6 (an enzyme required for fatty acid ω-hydroxylase). Thus, PPARα has an important role in the regulation of lipid metabolism and is part of the mechanism through which hypolipidimic compounds such as fibrates exert their effects. PPARγ is predominantly expressed in adipose tissue. Recently, a prostaglandin J2 metabolite, 15-Deoxy-D12,14-prostaglandin J2, has been identified as a potential physiological ligand of PPARγ. Both 15-Deoxy-D12,14-prostaglandin J2 treatment of preadipocytes or retroviral expression of PPARγ2 in fibroblasts induced adipocyte differentiation, demonstrating the role of PPARγ in adipocyte differentiation and lipid storage. The demonstration that anti-diabetic and lipid-lowering insulin sensitizing compounds known as thiazolidinediones are high affinity ligands for PPARγ suggests a broad therapeutic role for PPARγ ligands in the treatment of diabetes and disorders associated with insulin resistance (*e.g.* obesity and cardiovascular disease).

Nuclear receptor proteins contain a central DNA binding domain (DBD) and a COOH-terminal ligand binding domain (LBD). The DBD is composed of two highly conserved zinc fingers that target the receptor to specific promoter/enhancer DNA sequences known as hormone response elements (HREs). The LBD is about 200-300 amino acids in length and is less well conserved than the DBD. There are at least three functions for the LBD: dimerization, ligand binding, and transactivation. The transactivation function can be viewed as a molecular switch between a transcriptionally inactive and a transcriptionally active state of the receptor. Binding of a ligand which is an agonist flips the switch from the inactive state to the active state. The COOH-terminal portion of the LBD contains an activation function domain (AF2) that is required for the switch.

The ligand-induced nuclear receptor molecular switch is mediated through interactions with members of a family of nuclear receptor co-activators (*e.g.*, CBP/p300, SRC-1/NcoA-1, TIF2/GRIP-1/NcoA-2, and p/CIP). Upon binding of agonist to its cognate receptor LBD, a conformational change in the receptor protein creates a co-activator binding surface and results in recruitment of co-activator(s) to the receptor and subsequent transcriptional activation. The binding of antagonist ligands to nuclear receptors will not induce the required conformational change and prevents recruitment of co-activator and subsequent induction of transcription. The co-activators CREB-binding protein (CBP) and p300 are two closely related proteins that were originally discovered by virtue of their ability to interact with the transcription factor CREB. These two proteins share extensive amino acid sequence homology. CBP can form a bridge between nuclear receptors and the basic transcriptional machinery (Kamei et al., 1996, Cell 85:403-414; Chakravarti et al., 1996, Nature 383:99-103; Hanstein et al., 1996, Proc. Natl. Acad. Sci. USA 93:11540-11545; Heery et al., 1997, Nature 387:733-736). CBP also contains intrinsic histone acetyltransferase activity which could result in local chromatin rearrangement and further activation of transcription. Ligand- and AF2-dependent interaction between certain nuclear receptors and CBP has been demonstrated in *in vitro* pull down assays and far-western assays. This interaction is both necessary and sufficient for the transcriptional activation that is mediated by these nuclear receptors. Thus, an AF2 mutant of the estrogen receptor (ER) which abolishes the transcriptonal function of the receptor is incapable of interacting with CBP.

The N-termini of CBP and p300 have been shown to interact with the ligand-binding domains of some nuclear receptors (Kamei et al., 1996, Cell 85:403-414, hereinafter "Kamei"). Kamei was able to demonstrate direct interaction of CBP and p300 with nuclear receptors by several different methods:

(1) Kamei produced GST fusion proteins of the first 100 amino acids of the N-terminus of CBP. These fusion proteins were run out on a polyacrylamide gel, transferred to a membrane, and the membrane was exposed to ^{32}P-labeled ligand-binding domains of

nuclear receptors. In the presence of ligand, a specific binding interaction between the CBP and nuclear receptor fragments was detected in that the ^{32}P-labeled ligand-binding domains were observed to bind to the bands on the membrane containing the GST-CBP fusion proteins.

(2) Kamei also utilized the yeast two-hybrid system. The ligand-binding domain of the nuclear receptor fused to the DNA-binding domain of the LexA protein was used as bait. The amino terminal domain of CBP fused to the gal4 transactivation domain was used as prey. In the presence of ligand, a specific binding interaction (occurring *in vivo, i.e.*, within the yeast) was observed between the CBP and nuclear receptor fragments.

(3) Kamei observed ligand-induced binding between CBP and nuclear receptors via a gel-shift assay. This assay is based on the observation that, in the presence of ligand, nuclear receptors will bind to oligonucleotides containing their target recognition sequence. Such binding results in the formation of a nuclear receptor-ligand-oligonucleotide complex having a higher molecular weight than the oligonucleotide alone. This difference in molecular weight is detected via a shift in position of the ^{32}P-labeled oligonucleotide when it is run out on a polyacrylamide gel. Kamei found that a fragment of CBP (the N-terminal 100 amino acids) was capable of binding to the nuclear receptor-ligand-oligonucleotide complex and shifting the complex's position on the gel to an even higher molecular weight.

(4) Kamei was able to co-immunoprecipitate CBP using antibodies to nuclear receptors in extracts from a variety of cells in the presence of ligand.

(5) By the use of transcriptional activation assays, Kamei was able to demonstrate that nuclear receptors and CBP interact in a functional manner. Such transcriptional activation assays can indicate that two proteins are involved in a pathway that results in transcriptional activation but these assays do not prove that the interaction between the proteins is one of direct binding.

By the above-described methods, Kamei was able to demonstrate specific binding interactions between CBP and the retinoic acid receptor (RAR), glucocorticoid receptor (GR), thyroid hormone

receptor (T3R), and retinoid X receptor (RXR). Kamei also demonstrated specific binding between the N-terminus of p300 and RAR. However, Kamei did not demonstrate specific binding between CBP, p300, or any other nuclear receptor co-activators and PPARs.

What is striking about the methods used by Kamei is their extremely laborious and time consuming nature. Such methods involve, among other things, the construction of fusion proteins, the preparation of ^{32}P-labeled proteins, the construction of specialized expression vectors for the yeast two-hybrid assay and the transcriptional activation assays, the running of many gels, and the raising of antibodies. Most of these assays take days to carry out and preparing the reagents needed to carry them out may take weeks. Because of the complicated reagents that are involved in these assays and the time needed to prepare and run the assays, these assays tend to be costly. Investigators other than Kamei who have studied the interaction between nuclear receptors and CBP have also been forced to rely on such cumbersome methods (see, *e.g.*, Chakravarti et al., 1996, Nature 383:99-103; Hanstein et al., 1996, Proc. Natl. Acad. Sci. USA 93:11540-11545; Heery et al., 1997, Nature 387:733-736).

Kamei did not use the above-described methods to identify novel agonists or antagonists of nuclear receptors. The focus of Kamei was not on agonists or antagonists, but rather on the interaction between nuclear receptors and CBP. Although modifying the methods of Kamei to identify agonists or antagonists might be possible, such methods would suffer from serious disadvantages. This is because, as discussed above, all of the assays employed by Kamei to study the interaction of CBP and p300 with nuclear receptors are very laborious, slow, and costly. Given the therapeutic importance of steroid hormones such as estrogen, cortisol, progesterone, and other nuclear receptor agonists such as thyroid hormone and antidiabetic thiazolidinedione compounds, the need for improved high-throughput screening assays to identify potential pharmaceutical compounds affecting nuclear receptors is clear. Historically, therapeutically useful nuclear receptor ligand compounds were identified by screening animal models, an approach which is even more labor intensive and time consuming than the methods used by Kamei. Also, approaches such as those used by

Kamei are ill-suited for the identification of antagonists of nuclear receptors. It is now widely appreciated that antagonists of nuclear receptors can be valuable therapeutic agents. Examples of such therapeutically useful antagonists are tamoxifene, raloxifene, and RU-486.

What is needed is a high throughput, time and labor-saving, non-radioactive, inexpensive, and very reliable assay for the identification and characterization of both agonists and antagonists of nuclear receptors. Such an assay is provided by the present invention.

SUMMARY OF THE INVENTION

The present invention provides novel methods of identifying agonists and antagonists of nuclear receptors. The methods take advantage of the agonist-dependent binding of nuclear receptors and CBP, p300, or other nuclear receptor co-activators. In the absence of agonist, binding between the nuclear receptor and CBP, p300, or other nuclear receptor co-activators does not occur. If agonist is present, however, such binding occurs and can be detected by fluorescence resonance energy transfer (FRET) between a fluorescently-labeled nuclear receptor and fluorescently-labeled CBP, p300, or other nuclear receptor co-activator. Antagonists can be identified by virtue of their ability to prevent or disrupt the agonist-induced interaction of nuclear receptors and CBP, p300, or other nuclear receptor co-activators. In contrast to prior art methods of identifying agonists and antagonists of nuclear receptors, the methods of the present invention, are simple, rapid, and less costly.

The present invention provides a nuclear receptor or ligand binding domain thereof labeled with a fluorescent reagent for use in the above-described methods of identifying agonists and antagonists of nuclear receptors. The present invention also provides CBP, p300, or other nuclear receptor co-activator, or a binding portion thereof, labeled with a fluorescent reagent.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates a method of fluorescently labelling a protein or polypeptide with Europium cryptate (Eu3+K).

Figure 2 illustrates the format for experiments 1 and 2 of Table 1.

Figure 3 illustrates the format for experiment 3 of Table 1.

Figure 4 illustrates the format for experiment 4 of Table 1.

Figure 5 shows the results of studies using the methods of the present invention with four known PPARγ agonists. --o-- = AD5075; --□-- = Pioglitazone; --×-- = Troglitazone; --◊-- = BRL49653.

Figure 6 shows a measurement of the binding constant for the interaction between hCBP and PPARγ1LBD.

Figure 7A shows the amino acid sequence of human CBP (SEQ.ID.NO.:1).

Figure 7B shows the nucleotide sequence of a cDNA encoding human CBP (SEQ.ID.NO.:2). The open reading frame is at positions 76-1290.

Figure 8A shows the amino acid sequence of human PPARα (SEQ.ID.NO.:3).

Figure 8B shows the nucleotide sequence of a cDNA encoding human PPARα (SEQ.ID.NO.:4). The open reading frame is at positions 217-1623.

Figure 9A shows the amino acid sequence of human PPARγ1 (SEQ.ID.NO.:5).

Figure 9B shows the nucleotide sequence of a cDNA encoding human PPARγ1 (SEQ.ID.NO.:6). The open reading frame is at positions 173-1609.

Figure 10A shows the amino acid sequence of human PPARδ (SEQ.ID.NO.:7).

Figure 10B-C shows the nucleotide sequence of a cDNA encoding human PPARδ (SEQ.ID.NO.:8). The open reading frame is at positions 338-1663.

DETAILED DESCRIPTION OF THE INVENTION

For the purposes of this invention:

- an “agonist” is a substance that binds to nuclear receptors in such a way that a specific binding interaction between the nuclear receptor and CBP or other nuclear receptor co-activator can occur.

- an “antagonist” is a substance that is capable of preventing or disrupting the agonist-induced specific binding interaction between a nuclear receptor and CBP, p300, or another nuclear receptor co-activator.

- a “ligand” of a nuclear receptor is an agonist or an antagonist of the nuclear receptor.

- a “specific binding interaction,” “specific binding,” and the like, refers to binding between a nuclear receptor and CBP, p300, or other nuclear receptor co-activator which results in the occurrence of fluorescence resonance energy transfer between a fluorescent reagent bound to the nuclear receptor and a fluorescent reagent bound to CBP, p300, or other nuclear receptor co-activator.

With respect to CBP, p300, or other nuclear receptor co-activators, a “binding portion” is that portion of CBP, p300, or other nuclear receptor co-activators that is sufficient for specific binding interactions with nuclear receptors.

With respect to nuclear receptors, a “ligand binding domain” is that portion of a nuclear receptor that is sufficient to bind an agonist or antagonist of the nuclear receptor.

The present invention provides a high throughput, time and labor-saving, non-radioactive, inexpensive, and very reliable assay for the identification and characterization of both agonists and antagonists of nuclear receptors. In a general embodiment, the present invention provides methods of identifying agonists and antagonists for any nuclear receptor for which CBP, p300, or another nuclear receptor binding protein is a co-activator. Such agonists and antagonists are identified by virtue of their ability to induce or prevent binding between the ligand binding domain of a nuclear receptor and CBP, p300, or other nuclear receptor co-activator. The interaction between the nuclear receptor and CBP, p300, or other nuclear receptor co-activator is monitored by observing the occurrence of fluorescence resonance energy

transfer (FRET) between two fluorescent reagents. One fluorescent reagent is bound to the nuclear receptor; the other fluorescent reagent is bound to CBP, p300, or other nuclear receptor co-activator. The binding of fluorescent reagent to nuclear receptor, CBP, p300, or other nuclear receptor co-activator can be by a covalent linkage or a non-covalent linkage.

The present invention makes use of fluorescence resonance energy transfer (FRET). FRET is a process in which energy is transferred from an excited donor fluorescent reagent to an acceptor fluorescent reagent by means of intermolecular long-range dipole-dipole coupling. FRET typically occurs over distances of about 10è to 100è and requires that the emission spectrum of the donor reagent and the absorbance spectrum of the acceptor reagent overlap adequately and that the quantum yield of the donor and the absorption coefficient of the acceptor be sufficiently high. In addition, the transition dipoles of the donor and acceptor fluorescent reagents must be properly oriented relative to one another. For a review of FRET and its applications to biological systems, see Clegg, 1995, Current Opinions in Biotechnology 6:103-110.

The present invention makes use of a nuclear receptor or ligand binding domain thereof labeled with a first fluorescent reagent and CBP, p300, or other nuclear receptor co-activator, or a binding portion thereof, labeled with a second fluorescent reagent. The second fluorescent reagent comprises a fluorophore capable of undergoing energy transfer by either (a) donating excited state energy to the first fluorescent reagent, or (b) accepting excited state energy from the first fluorescent reagent. In other words, according to the present invention, either the first or the second fluorescent reagents can be the donor or the acceptor during FRET.

The first and second fluorescent reagents are spectropscopically complementary to each other. This means that their spectral characteristics are such that excited state energy transfer can occur between them. FRET is highly sensitive to the distance between the first and second fluorescent reagents. For example, FRET varies inversely with the sixth power of the distance between the first and second fluorescent reagents. In the absence of agonist, the first

fluorescent reagent, bound to the nuclear receptor or ligand binding domain thereof, will not be near the second fluorescent reagent, bound to CBP, p300, or other nuclear receptor co-activator, or binding portion thereof. Thus, no FRET, or very little FRET, will be observed. In the presence of agonist, however, interaction between the nuclear receptor and CBP, p300, or other nuclear receptor co-activator will occur, thus bringing close together the first and the second fluorescent reagents, allowing FRET to occur and be observed.

Accordingly, the present invention provides a method of identifying an agonist of a nuclear receptor that comprises providing:

(a) a nuclear receptor or ligand binding domain thereof labeled with a first fluorescent reagent;

(b) CBP, p300, or other nuclear receptor co-activator, or a binding portion thereof, labeled with a second fluorescent reagent; and

(c) a substance suspected of being an agonist of the nuclear receptor;

under conditions such that, if the substance is an agonist of the nuclear receptor, binding between the nuclear receptor or ligand binding domain thereof and CBP, p300, or other nuclear receptor co-activator, or a binding portion thereof, will occur; and

(d) measuring fluorescence resonance energy transfer (FRET) between the first and second fluorescent reagents;

where the occurrence of FRET indicates that the substance is an agonist of the nuclear receptor.

In particular embodiments, the nuclear receptor is selected from the group consisting of steroid receptors, thyroid hormone receptors, retinoic acid receptors, peroxisome proliferator-activated receptors, retinoid X receptors, glucocorticoid receptors, vitamin D receptors, and "orphan nuclear receptors" such as LXR, FXR, etc.

In a particular embodiment, the nuclear receptor or ligand binding domain thereof is a full-length nuclear receptor. In another embodiment, the nuclear receptor or ligand binding domain thereof is a ligand binding domain of a nuclear receptor. In another embodiment, the nuclear receptor or ligand binding domain thereof comprises an AF-2 site of a nuclear receptor.

In a particular embodiment, the nuclear receptor or ligand binding domain thereof is a full-length PPAR. In another embodiment, the nuclear receptor or ligand binding domain thereof is the ligand binding domain of a PPAR. In a further embodiment, the PPAR is selected from the group consisting of PPARα, PPARγ1, PPARγ2, and PPARδ. In a further embodiment, the ligand binding domain of the PPAR contains amino acid residues 176-478 of human PPARγ1.

In a particular embodiment, the nuclear receptor or ligand binding domain thereof contains amino acids 143-462 of human RARα. In another embodiment, the nuclear receptor or ligand binding domain thereof contains amino acids 122-410 of rat $T_3R\alpha 1$. In another embodiment, the nuclear receptor or ligand binding domain thereof contains amino acids 227-463 of mouse RXRγ. In another embodiment, the nuclear receptor or ligand binding domain thereof contains amino acids 251-595 of human ER.

In a particluar embodiment, the above-described methods utilize full-length CBP, either mouse or human. In other embodiments, the methods utilize amino acid residues 1-113 of human CBP. In another embodiment, the methods utilize amino acid residues 1-453 of human CBP.

The conditions under which the methods described above are carried out are conditions that are typically used in the art for the study of protein-protein interactions: *e.g.*, physiological pH; salt conditions such as those represented by such commonly used buffers as PBS; a temperature of about 4°C to about 55°C. The presence of commonly used non-ionic detergents, *e.g.*, NP-40®, sarcosyl, Triton X-100®, is optional. When europium cryptates are used as fluorescent reagents, reactions should contain KF at a concentration of at least 200 mM.

Heery et al., 1997, Nature 387:733-736 showed that interactions between nuclear receptors and a variety of nuclear receptor co-activators are mediated by a short amino acid sequence in the nuclear receptor co-activators having the amino acid sequence LXXLL, where L is leucine and X represents any amino acid. Accordingly, the present invention can be practiced with a binding portion of a nuclear receptor co-activator, provided that the binding portion contains the amino acid

sequence LXXLL. Therefore, the present invention includes a method of identifying an agonist of a nuclear receptor that comprises providing:

(a) a nuclear receptor or ligand binding domain thereof labeled with a first fluorescent reagent;

(b) a binding portion of a nuclear receptor co-activator, where the binding portion contains the amino acid sequence LXXLL, and where the binding portion is labeled with a second fluorescent reagent; and

(c) a substance suspected of being an agonist of the nuclear receptor;

under conditions such that, if the substance is an agonist of the nuclear receptor, binding between the nuclear receptor or ligand binding domain thereof and the binding portion of the nuclear receptor co-activator will take place; and

(d) measuring fluorescence resonance energy transfer (FRET) between the first and second fluorescent reagents;

where the occurrence of FRET indicates that the substance is an agonist of the nuclear receptor.

In a particular embodiment, the nuclear receptor co-activator is selected from the group consisting of: human RIP-140, human SRC-1, mouse TIF-2, human or mouse CBP, human or mouse p300, mouse TIF-1, and human TRIP proteins.

In a particular embodiment, the nuclear receptor co-activator is human RIP-140 and the binding portion includes a contiguous stretch of amino acids of human RIP-140 selected from the group consisting of: positions 20-29, 132-139, 184-192, 266-273, 379-387, 496-506, 712-719, 818-825, 935-944, and 935-942.

In another embodiment, the nuclear receptor co-activator is human SRC-1 and the binding portion includes a contiguous stretch of amino acids of human SRC-1 selected from the group consisting of: positions 45-53, 632-640, 689-696, 748-755, and 1434-1441.

In another embodiment, the nuclear receptor co-activator is mouse TIF-2 and the binding portion includes a contiguous stretch of amino acids of mouse TIF-2 selected from the group consisting of: positions 640-650, 689-699, and 744-754.

In another embodiment, the nuclear receptor co-activator is human or mouse CBP and the binding portion includes a contiguous stretch of amino acids of human or mouse CBP selected from the group consisting of: positions 68-78 and 356-366.

In another embodiment, the nuclear receptor co-activator is human or mouse p300 and the binding portion includes a contiguous stretch of amino acids of human or mouse p300 selected from the group consisting of: positions 80-90 and 341-351.

In another embodiment, the nuclear receptor co-activator is mouse TIF-1 and the binding portion includes a contiguous stretch of amino acids of mouse TIF-1 containing positions 722-732.

In another embodiment, the nuclear receptor co-activator is human TRIP2 and the binding portion includes a contiguous stretch of amino acids of human TRIP2 containing positions 23-33.

In another embodiment, the nuclear receptor co-activator is human TRIP3 and the binding portion includes a contiguous stretch of amino acids of human TRIP3 containing positions 97-107.

In another embodiment, the nuclear receptor co-activator is human TRIP4 and the binding portion includes a contiguous stretch of amino acids of human TRIP4 containing positions 36-46.

In another embodiment, the nuclear receptor co-activator is human TRIP5 and the binding portion includes a contiguous stretch of amino acids of human TRIP5 containing positions 26-36.

In another embodiment, the nuclear receptor co-activator is human TRIP8 and the binding portion includes a contiguous stretch of amino acids of human TRIP8 containing positions 36-46.

In another embodiment, the nuclear receptor co-activator is human TRIP9 and the binding portion includes a contiguous stretch of amino acids of human TRIP9 selected from the group consisting of: positions 73-83, 256-266 and 288-298.

For amino acid sequences of nuclear receptor co-activators, see Yao et al., 1996, Proc. Natl. Acad. Sci. USA 93:10626-10631 (SRC-1); O§ate et al., 1995, Science 270:1354-1357 (SRC-1); Cavaillès et al., 1995, EMBO J. 14:3741-3751 (RIP-140); Voegel et al., 1996, EMBO J. 15:101-108 (TIF-2); Kwok et al., 1994, Nature 370:223-226 (CBP); Arias et al., 1994, Nature 370:226-229 (CBP); Eckner et al., 1994, Genes Dev. 8:869-884

(p300); Le Douarin et al., 1995, EMBO J. 14:2020-2033 (TIF-1); Lee et al., 1995, Nature 374:91-94 (TRIP proteins).

The particular embodiments of the present invention described above are all particular embodiments of a more general method that is also part of the present invention. That general method is a method of identifying an agonist of a nuclear receptor that comprises providing:

(a) a nuclear receptor or ligand binding domain thereof labeled with a first fluorescent reagent;

(b) a polypeptide containing the amino acid sequence LXXLL where the polypeptide is labeled with a second fluorescent reagent; and

(c) a substance suspected of being an agonist of the nuclear receptor;

under conditions such that, if the substance is an agonist of the nuclear receptor, binding between the nuclear receptor or ligand binding domain thereof and the polypeptide will take place; and

(d) measuring fluorescence resonance energy transfer (FRET) between the first and second fluorescent reagents;

where the occurrence of FRET indicates that the substance is an agonist of the nuclear receptor.

In a particular embodiment, the amino acid sequence LXXLL is present in an α helical portion of the polypeptide. In another embodiment, the amino acid sequence LXXLL is present in an α helical portion of the polypeptide and the leucines form a hydrophobic face.

The present invention provides methods for identifying antagonists of a nuclear receptor. Such methods are based on the ability of the antagonist to prevent the occurrence of agonist-induced binding between a nuclear receptor and CBP, p300, or other nuclear receptor co-activator, or to disrupt such binding after it has occurred. Thus, the present invention provides a method for identifying antagonists of nuclear receptors that comprises providing:

(a) a nuclear receptor or ligand binding domain thereof labeled with a first fluorescent reagent;

(b) CBP, p300, or other nuclear receptor co-activator, or a binding portion thereof, labeled with a second fluorescent reagent;

(c) an agonist of the nuclear receptor; and

(d) a substance suspected of being an antagonist of the nuclear receptor;

under conditions such that, in the absence of the substance, binding between the nuclear receptor or ligand binding domain thereof and CBP, p300, or other nuclear receptor co-activator, or a binding portion thereof will occur; and

(e) measuring fluorescence resonance energy transfer (FRET) between the first and second fluorescent reagents when the substance is present and measuring FRET between the first and second fluorescent reagents when the substance is absent;

where the a decrease in FRET when the substance is present indicates that the substance is an antagonist of the nuclear receptor.

In particular embodiments, the nuclear receptor is selected from the group consisting of steroid receptors, thyroid hormone receptors, retinoic acid receptors, peroxisome proliferator-activated receptors, retinoid X receptors, glucocorticoid receptors, vitamin D receptors, and "orphan nuclear receptors" such as LXR, FXR, etc.

In a particular embodiment, the nuclear receptor or ligand binding domain thereof is a full-length nuclear receptor. In another embodiment, the nuclear receptor or ligand binding domain thereof is a ligand binding domain of a nuclear receptor. In another embodiment, the nuclear receptor or ligand binding domain thereof is an AF-2 site of a nuclear receptor.

In a particular embodiment, the nuclear receptor or ligand binding domain thereof is a full-length PPAR. In another embodiment, the nuclear receptor or ligand binding domain thereof is the ligand binding domain of a PPAR. In a further embodiment, the PPAR is selected from the group consisting of PPARα, PPARγ, and PPARδ. In a further embodiment, the ligand binding domain of the PPAR contains amino acid residues 176-478 of human PPARγ1.

In a particular embodiment, the nuclear receptor or ligand binding domain thereof contains amino acids 143-462 of human RARα. In another embodiment, the nuclear receptor or ligand binding domain thereof contains amino acids 122-410 of rat $T_3R\alpha1$. In another

embodiment, the nuclear receptor or ligand binding domain thereof contains amino acids 227-463 of mouse RXRγ. In another embodiment, the nuclear receptor or ligand binding domain thereof contains amino acids 251-595 of human ER.

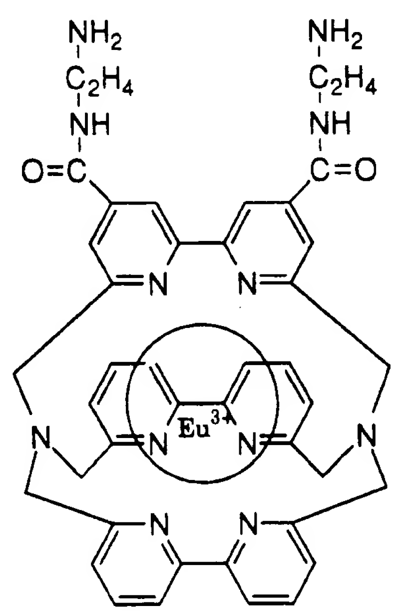
In a particular embodiment, the above-described methods utilize full-length CBP, either mouse or human. In other embodiments, the methods utilize amino acid residues 1-113 of human CBP. In another embodiment, the methods utilize amino acid residues 1-453 of human CBP.

The conditions under which the methods described above are carried out are conditions that are typically used in the art for the study of protein-protein interactions: *e.g.*, physiological pH; salt conditions such as those represented by such commonly used buffers as PBS; a temperature of about 4°C to about 55°C. The presence of commonly used non-ionic detergents, *e.g.*, NP-40®, sarcosyl, Triton X-100®, is optional. When europium cryptates are used as fluorescent reagents, reactions should contain KF at a concentration of at least 200 mM.

In principle, one could measure FRET by monitoring either (a) a decrease in the emission of the donor fluorescent reagent following stimulation at the donor's absorption wavelength and/or (b) an increase in the emission of the acceptor reagent following stimulation at the donor's absorption wavelength. In practice, FRET is most effectively measured by emission ratioing. Emission ratioing monitors the change in the ratio of emission by the acceptor over emission by the donor. An increase in this ratio signifies that energy is being transferred from donor to acceptor and thus that FRET is occurring. Emission ratioing can be measured by employing a laser-scanning confocal microscope. Emission ratioing is preferably done by splitting the emitted light from a sample with a dichroic mirror and measuring two wavelength bands (corresponding to the donor and the acceptor emission wavelengths) simultaneously with two detectors. Alternatively, the emitted light can be sampled consecutively at each wavelength (by using appropriate filters) with a single detector. In any case, these and other methods of measuring FRET are well known in the art.

Although a variety of donor and acceptor fluorescent reagents can be used in the practice of the present invention, preferred embodiments of the present invention make use of cryptates of fluorescent reagents as donor reagents. Inclusion of a substrate into the intramolecular cavity of a macropolycyclic ligand results in the formation of a cryptate. The macropolycyclic ligand shields the substrate from interaction with solvent and other solute molecules. If the substrate is a fluororescent reagent, formation of a cryptate may result in markedly different spectroscopic characteristics for the reagent as compared to the spectroscopic characteristics of the free reagent.

The present invention includes the use of europium (Eu^{III}) or terbium (Tb^{III}) cryptates as donor fluorescent reagents. Such Eu^{III} or Tb^{III} cryptates, as well as methods for their formation, are well known in the art. For example, see Alpha et al., 1987, Angew. Chem. Int. Ed. Engl. 26:266-267; Mathis, 1995, Clin. Chem. 41:1391-1397. A europium cryptate is formed by the inclusion of a europium ion into the intramolecular cavity of a macropolycyclic ligand which contains bipyridine groups as light absorbers. When europium cryptates are present in solution together with fluoride ions, a total shielding of the europium cryptate fluorescence is occurs. The molecular structure of a europium cryptate is shown below.



Europium cryptates can be conjugated to proteins by the use of well-known heterobifunctional reagents (see, *e.g.*, International Patent Application WO 89/05813; Prat et al., 1991, Anal. Biochem. 195:283-289; Lopez et al., 1993, Clin. Chem. 39:196-201).

The present invention includes the use of XL665 as the acceptor fluorescent reagent. XL665 is a crosslinked derivative of allophycocyanin (APC). APC is a porphyrin containing protein which is derived from the light harvesting system of algae (Kronick, 1986, M. Immunol. Meth. 92:1-13). XL665 has an absorption maximum at ≈620 nm and an emission maximum at 665 nm. In some embodiments of the invention, XL665 is labeled with streptavidin in order to effect the binding of the streptavidin-labled XL665 to a biotin-labeled substance, *e.g.*, CBP or the ligand binding domain of a nuclear receptor. Streptavidin labeling of XL655 and biotin labeling of CBP, or the ligand binding domain of a nuclear receptor, can be performed by well known methods.

In a preferred embodiment of the invention, XL665 as the acceptor fluorescent reagent is combined with Europium cryptate (Eu3+K) as the donor fluorescent reagent. Europium cryptate (Eu3+K) has a large Stokes shift, absorbing light at 337 nm and emitting at 620 nm. Thus, the emission maximum of Europium cryptate (Eu3+K) overlaps the absorption maximum of XL665. Europium cryptate (Eu3+K) has a large temporal shift; the time between absorption and emission of a photon is about 1 millisecond. This is advantageous because most background fluorescence signals in biological samples are short-lived. Thus the use of a fluorescent reagent such as europium cryptate, with a long fluorescent lifetime, permits time-resolved detection resulting in the reduction of background interference.

The spectral and temporal properties of europium cryptate (Eu3+K) result in essentially no fluorescence background and thus assays using this fluorescent reagent can be carried out in a "mix and read" mode, greatly facilitating its use as a high throughput screening tool. For the embodiment using Europium cryptate (Eu3+K) and XL665, the measuring instrument irradiates the sample at 337 nm and measures the fluorescence output at two wavelengths, 620 nm (B counts, europium fluorescence) and 665 nm (A counts, XL665 fluorescence).

The extent of flurorescent resonance energy transfer is measured as the ratio between these two values. Typically this ratio is multiplied by 10,000 to give whole numbers.

Other FRET donor-acceptor pairs are suitable for the practice of the present invention. For example, the following donor-acceptor pairs can be used: dansyl/fluorescein; fluorescein/rhodamine; tryptophan/aminocoumarin.

The present invention provides a nuclear receptor or ligand binding domain thereof labeled with a fluorescent reagent for use in the above-described methods of identifying agonists and antagonists of nuclear receptors. The present invention also provides CBP, p300, or other nuclear receptor co-activator, or a binding portion thereof, labeled with a fluorescent reagent.

In a particular embodiment, the nuclear receptor or ligand binding domain thereof is selected from the group consisting of PPARα, PPARγ, PPARδ, a ligand binding domain of PPARα, PPARγ, or PPARδ, and amino acid residues 176-478 of human PPARγ1 and the fluorescent reagent is selected from the group consisting of XL665 and Europium cryptate (Eu3+K).

In a particular embodiment, CBP, p300, or other nuclear receptor co-activator is labeled with a fluorescent reagent selected from the group consisting of XL665 and Europium cryptate (Eu3+K).

The following non-limiting examples are presented to better illustrate the invention.

EXAMPLE 1

Cloning, expression, and purification of human CBP and PPAR proteins

To test whether human CBP can interact with PPARs in an agonist-dependent manner, we cloned the human cDNA fragments encoding the NH_2-terminal 1-113 amino acids (hCBP1-113) and 1-453 amino acids (hCBP1-453) of human CBP by the polymerase chain reaction (PCR). The DNA and amino acid sequences of human CBP are

disclosed in Borrow et al., 1996, Nature Genet. 14:33-41 and in GenBank, accession no. U47741.

The primers used for hCBP1-113 were:
5'-ACTCGGATCCAAGCCATGGCTGAGAACTTGCTGGACGG-3' (SEQ.ID.NO.:9) and
5'-CACAAAGCTTAGGCCATGTTAGCACTGTTCGG-3' (SEQ.ID.NO.: 10).
These primers were expected to amplify a 0.9 kb DNA fragment.

The primers for hCBP1-453 were:
5'-ACTCGGATCCAAGCCATGGCTGAGAACTTGCTGGACGG-3' (SEQ.ID.NO.:9) and
5'CTCAGTCGACTTATTGAATTCCACTAGCTGGAGATCC-3' (SEQ.ID.NO.:11).
These primers were expected to amplify a 1.5 kb DNA fragment..

The template for the PCR reaction was a human fetal brain cDNA library (Stratagene, Catalogue #IS 937227). Of course, any human cDNA library from a tissue expressing CBP could have been used. The PCR amplified 0.9 kb and 1.5 kp DNA fragments which were digested with restriction endonucleases and ligated into pBluescript II vector. DNA sequencing analysis confirmed that the amplified fragments were identical to the corresponding published nucleic acid sequences of human CBP.

Based on the publicly available sequences for human CBP cited above, other primers could be readily identified and prepared by those skilled in the art in order to amplify and clone other portions of cDNA encoding human CBP from appropriate cDNA libraries. Once such portions of human CBP are produced, they could be used in the methods of the present invention in a manner similar to that described herein for hCBP1-113 and hCBP1-453. The amino acid sequence of human CBP is shown in Figure 7A; the nucleic acid sequence of the cDNA encoding human CBP is shown in Figure 7B.

To express the polypeptides encoded by the PCR fragments, vectors encoding fusion proteins of the polypeptides and glutathione S-transferase (GST) were constructed and expressed in *E. coli*. The PCR fragments were subcloned into the expression vector pGEX (Pharmacia Biotech) to generate pGEXhCBP1-113 and pGEXhCBP1-453.

pGEXhCBP1-113 and pGEXhCBP1-453 were transfected into the DH5α strain of *E. coli* (GIBCO BRL) and the bacteria hosting either pGEXhCBP1-113 or pGEXhCBP1-453 were cultured in LB medium (GIBCO BRL) to a density of OD_{600} = 0.7-1.0 and induced for overexpression of the GST-CBP fusion proteins by addition of IPTG (isopropylthio-β-galactoside) to a final concentration of 0.2 mM. The IPTG induced cultures were further grown at room temperature for 2-5 hrs. The cells were harvested by centrifugation for 10 min at 5000g. The cell pellet was used for GST-CBP fusion protein purification by following the procedure from Pharmacia Biotech using Glutathione Sepharose beads. hCBP1-113 and hCBP1-453 proteins were generated by cleaving the corresponding GST fusion proteins with thrombin. SDS-polyacrylamide gel electrophoresis analysis showed that the preparation from pGEXhCBP1-113 gave two polypeptide bands, with apparent molecular weight of 12 kd and 10 kd. The 12 kd band is the expected size of hCBP1-113 and the 10 kd band is most likely a premature translational termination product. The preparation from pGEXhCBP1-450 gave a single band with the expected size, 50 kd.

cDNAs encoding full-length PPARα and PPARγ1 were subcloned into pGEX vectors for the production of GST-PPARα and GST-PPARγ1 fusion proteins in *E.coli*. PPARγ1 was cloned from a human fat cell cDNA library (see Elbrecht et al., 1996, Biochem. Biophys. Res. Comm. 224:431-437). A cDNA encoding the human PPARγ1 ligand binding domain (PPARγ1LBD; amino acids 176-478 of PPARγ1) was subcloned from a modified pSG5 vector as a Xho I (site located in the N-terminus of the LBD)/ Xba I (site located in the pSG5 vector) fragment. The Xba I site was blunt-ended with T4 DNA polymerase. The 1.1 kb fragment containing the LBD was purified from an agarose gel and ligated into pGEX-KG (see Guan & Dixon, 1991, Anal. Biochem. 192:262-267) that had been digested with Xho I and Hind III (the Hind III site had been blunt-ended with T4 DNA polymerase). This construct was used for the production of GST-hPPARγ1LBD and hPPARγ1LBD (the ligand binding domain cleaved free of GST). The overexpression and purification of PPARα, PPARγ1, and PPARγ1LBD were as described above for CBP.

The DNA and amino acid sequences of human PPARα are disclosed in Schmidt et al., 1992, Mol. Endocrinol. 6:1634-1641 and in GenBank, accession no. L07592. See Figure 8A and 8B.

The DNA and amino acid sequences of human PPARγ1 are disclosed in Greene et al., 1995, Gene Expr. 4:281-299; Qi et al., 1995, Mol. Cell. Biol. 15:1817-1825; Elbrecht et al., 1996, Biochem. Biophys. Res. Comm. 224:431-437; and in GenBank, accession no. L40904. See Figure 9A and 9B. Human PPARγ2 contains the same amino acid sequence as human PPARγ1 except for an amino terminal addition of 24 amino acids (see Elbrecht et al., 1996, Biochem. Biophys. Res. Comm. 224:431-437). Thus, the amino acid sequence of the ligand binding domain of human PPARγ2 is the same as the amino acid sequence of the ligand binding domain of human PPARγ1, although the numbering of the amino acids differs (176-478 for human PPARγ1 and 200-502 for human PPARγ2).

The DNA and amino acid sequences of human PPARδ are disclosed in Sher et al., 1993, Biochemistry 32:5598-5604 and in GenBank, accession no. L02932. See Figure 10A-C.

EXAMPLE 2

Interaction between PPARs and hCBP fragments

Experiments were first conducted using hCBP1-113 and hPPARγ1LBD. Purified hPPARγ1LBD was biotinylated with Sulfo-NHS-LC-Biotin (PIERCE) to a biotin:hPPARγ1LBD ratio of 3:1 according to the procedure provided by PIERCE. Purified hCBP1-113 was directly labeled with europium cryptate (Eu3+K) by the method illustrated in Figure 1. Biotin-labeled hPPARγ1LBD, Eu3+K-labeled hCBP1-113, and streptavidin-labeled XL665 (SA-XL665; from PACKARD) were incubated together in the presence or absence of 1 μM of known PPARγ agonist (BRL49653 or AD5075).

Thus, this experimental format made use of the fluorescent reagent pair europium cryptate (Eu3+K), which acted as donor, and XL665, which acted as acceptor. hCBP1-113 was directly labeled with europium cryptate (Eu3+K); hPPARγ1LBD was indirectly labeled with XL665 by means of a biotin-streptavidin link. The emission maximum

of europium cryptate (Eu3+K) overlaps with the absorption maximum of XL665. Therefore, when europium cryptate (Eu3+K) and XL665 are in close proximity, and the sample is illuminated with light at 337 nm (the absorption maximum of europium cryptate (Eu3+K)), FRET can occur between europium cryptate (Eu3+K) and XL665. This FRET manifests itself as increased emission at 665 nm by XL665. Figure 2 shows a schematic of the format used in this experiment (experiment 1 of Table 1). When agonist is bound to hPPARγ1LBD, a specific interaction occurs between hPPARγ1LBD and hCBP1-113, thus bringing europium cryptate (Eu3+K) and XL665 into close enough proximity for FRET to occur. In the absence of agonist, no interaction occurs between hPPARγ1LBD and hCBP1-113 and thus europium cryptate (Eu3+K) and XL665 are not brought into close proximity and no FRET occurs. When FRET occurs, the amount of light given off by the sample at the emission maximum of XL665 (665 nm) is increased relative to the amount of light given off by the sample at the emission maximum of europium cryptate (Eu3+K) (620 nm). Therefore, measuring the ratio of emission at 665 nm to 620 nm in the presence and the absence of a substance suspected of being an agonist allows for the determination of whether that substance actually is an agonist. If the substance is an agonist, an increase in the ratio of emission at 665 nm to 620 nm in the presence of the substance will be observed.

Reactions were carried out in microtiter plates. Reaction conditions were: appropriate volume (total 250 μl) of the reaction buffer (either PBS or HEPES, see below, containing 500 mM KF, 0.1% bovine serum albumin, BSA) was added to each well, followed by addition of ligands (BRL49653 or AD5075 at a final concentration of 1 μM and 0.1% dimethylsulfoxide (DMSO) or vehicle control (0.1% DMSO), Eu3+K labeled hCBP (100 nM), biotin-hPPARγ1LBD (100 nM), and streptavidin-labeled XL665 (100 nM) to appropriate wells. After mixing, 200 μl of reaction mixture was transferred to a new well. The plate was either directly measured for fluorescence resonance energy transfer (FRET) or covered with sealing tape (PACKARD) to avoid evaporation and incubated at room temperature for up to 24 hrs before measuring FRET.

The results of this experiment and others described below yielded ratio values as follows:

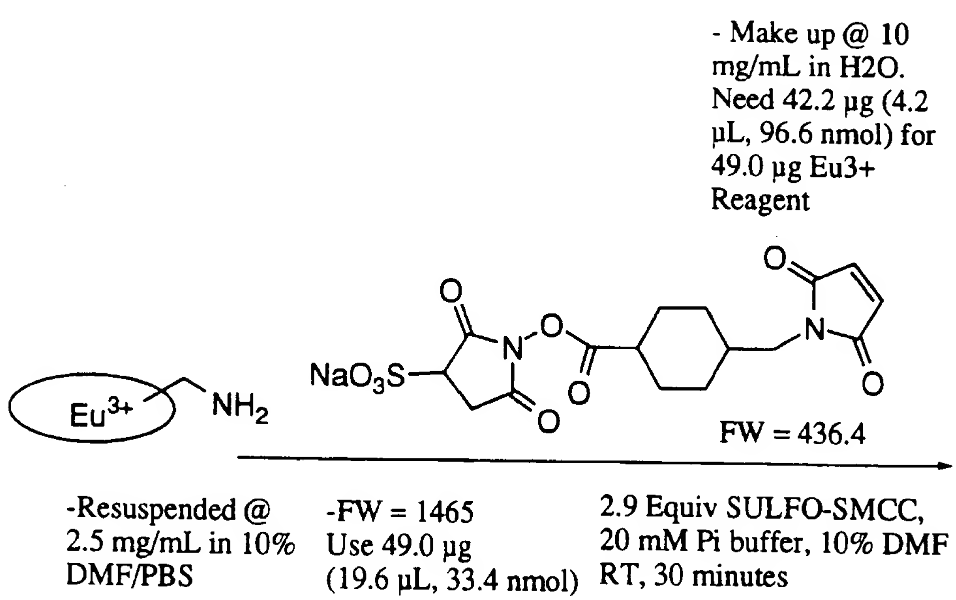
Table 1

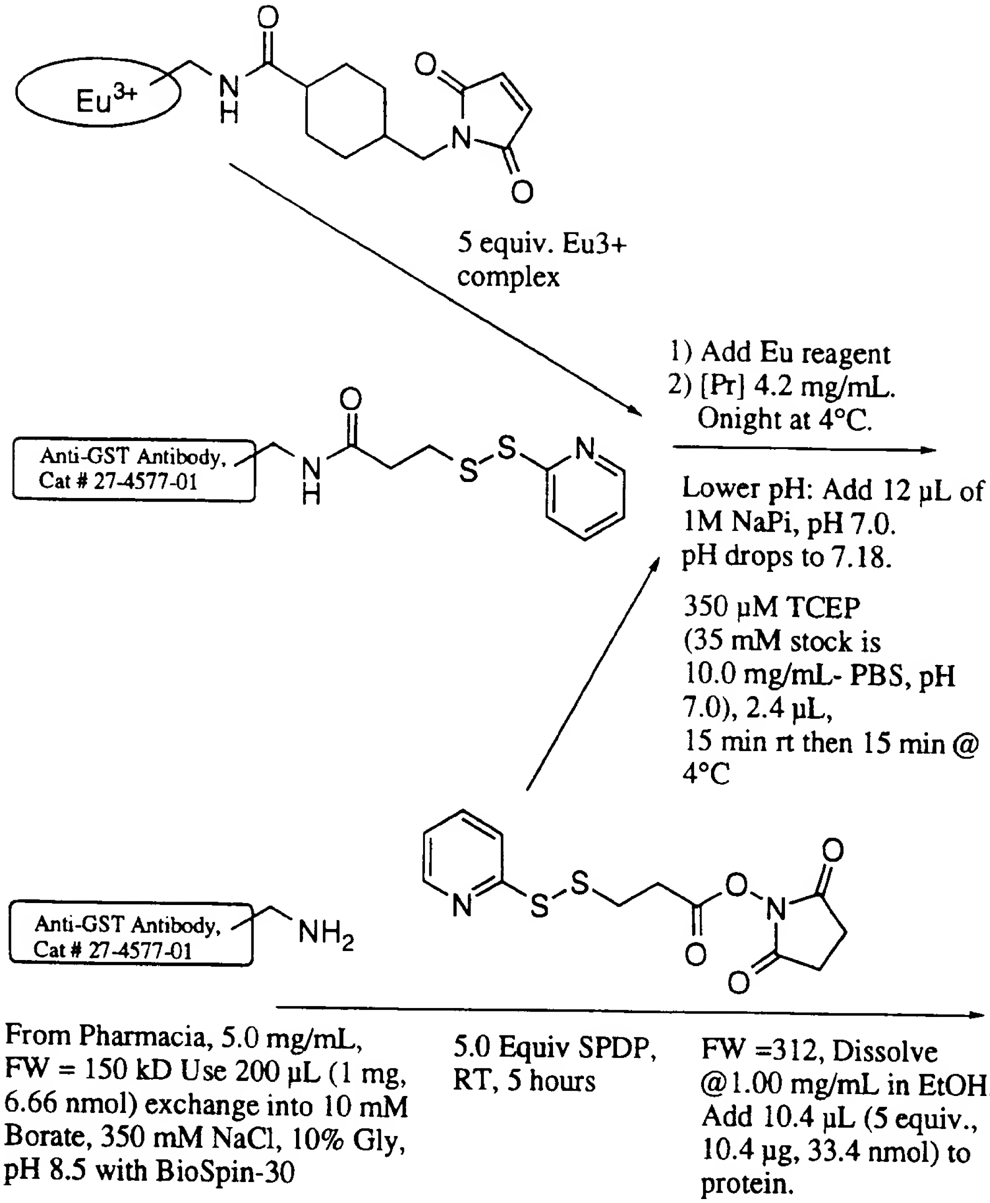
Experiment	Buffer	Emission ratio with AD5075	Emission ratio with vehicle
1	PBS	1134	1074
2	HEPES + 0.05% NP40	967	617
3	HEPES + 0.05% NP40	1078	536
4	HEPES + 0.05% CHAPS	1883	487

Experiment 1 of Table 1 was carried out using PBS (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.4 mM KH_2PO_4, pH 7.4). The greater emission ratio observed in the presence of AD5075 demonstrated that a specific interaction between hCBP1-113 and hPPARγ1LBD occurred in the presence of the agonist AD5075. Although it was clear that FRET was occurring, the signal-noise ratio was small. In experiment 2 of Table 1, HEPES buffer (N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid, 100 mM, pH 7.0) containing 0.05% NP40 (Nonidet P-40) was used instead of PBS and an improved signal-noise ratio was obtained.

In order to get an even better signal-noise ratio, the above-described format was modified slightly for experiment 3. In experiment 3, SA-XL665 (500 nM), biotin-labeled hPPARγ1LBD (100 nM), GST-hCBP1-113, and Eu3+K labeled anti-GST antibody (2.5 μl) were incubated in the presence or absence of AD5075 (1 μM) in HEPES buffer containing 0.05% NP40. A two-fold signal- noise ratio was obtained. Figure 3 shows a schematic of the format used in experiment 3.

The anti-GST antibody was a goat antibody to GST from Pharmacia (catalogue number 27-4577-01) that was labeled with Eu3+K according to the procedure summarized below.





To further improve the signal to noise ratio, a series of experiments were conducted. Experiment 4 of Table 1 exemplifies results obtained from those efforts. cDNA encoding a longer fragment of hCBP was cloned and expressed to get hCBP1-453. hCBP1-453 was biotinylated. Biotin-labeled hCBP1-453 (25 nM), SA-XL665 (100 nM), GST-hPPARγ1LBD (1 nM), and Eu3+K-labeled anti-GST antibody (2 nM) were mixed together in the presence or absence of 1 µM AD5075. The detergent was changed from 0.05% NP40 to 0.5% CHAPS (3-{[3-cholamidopropyl]dimethyl-ammoniol}-1-propanesulfonate). A three- to

four-fold signal-noise ratio was obtained. Figure 4 shows the strategy used for experiment 4 and similar experiments.

The correlation between results from the above-described assays and previously reported results from *in vitro* binding and transcriptional activation assays of selected antidiabetic insulin sensitizers that are known to be PPARγ agonists (Elbrecht et al., 1996, Biochem Biophys Res Comm 224:431-437) was analyzed by titrating those known PPARγ agonists in the assays described above and comparing EC_{50}s so obtained with previously described values for potency in binding or transcriptional activation assays for the known agonists. The results are shown in Figure 5. From Figure 5, the following EC_{50}s can be derived:

AD5075 = 8 nM
BRL49653 = 53 nM
Troglitazone = 646 nM
Pioglitazone = 890 nM.

These EC_{50}s generated in the above-described assays are in close agreement with those generated by *in vitro* binding and transcriptional activation studies (Elbrecht et al., 1996, Biochem Biophys Res Comm 224:431-437).

The above-described assay can also be used to characterize the interaction between nuclear receptors with co-activators as, *e.g.*, by determining the binding constant for that interaction. Figure 6 shows an example of such an application. Saturating amounts of PPARγ agonist (10 μM BRL49653) were used. Increasing concentrations of non-biotinylated hCBP1-453 were used to titrate away biotin-hCBP-PPARγ1LBD complex and decrease the fluorescence energy transfer. A Kd of 300 nM for the interaction between hCBP1-453 and PPARγ1LBD can be derived from the results illustrated in Figure 6 and this Kd (300 nM) is a measurement ofthe affinity between CBP and PPARγ.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties.

WHAT IS CLAIMED:

1. A method of identifying an agonist of a nuclear receptor that comprises providing:

(a) a nuclear receptor or ligand binding domain thereof labeled with a first fluorescent reagent;

(b) CBP, p300, or other nuclear receptor co-activator, or a binding portion thereof, labeled with a second fluorescent reagent; and

(c) a substance suspected of being an agonist of the nuclear receptor;

under conditions such that, if the substance is an agonist of the nuclear receptor, binding between the nuclear receptor or ligand binding domain thereof and CBP, p300, or other nuclear receptor co-activator, or a binding portion thereof, will occur; and

(d) measuring fluorescence resonance energy transfer (FRET) between the first and second fluorescent reagents;

where the occurrence of FRET indicates that the substance is an agonist of the nuclear receptor.

2. The method of claim 1 where the nuclear receptor or ligand binding domain thereof is selected from the group consisting of steroid receptors, thyroid hormone receptors, retinoic acid receptors, peroxisome proliferator-activated receptors, retinoid X receptors, glucocorticoid receptors, vitamin D receptors, LXR, and FXR.

3. The method of claim 1 where the nuclear receptor or ligand binding domain thereof is selected from the group consisting of a full-length nuclear receptor, a ligand binding domain of a nuclear receptor, and an AF-2 site of a nuclear receptor.

4. The method of claim 1 where the nuclear receptor or ligand binding domain thereof comprises an AF-2 site of a nuclear receptor.

5. The method of claim 1 where the nuclear receptor or ligand binding domain thereof is selected from the group consisting of a

full-length PPAR, a ligand binding domain of a PPAR, and amino acid residues 176-478 of human PPARγ1.

6. The method of claim 1 where the nuclear receptor or ligand binding domain thereof is selected from the group consisting of PPARα, PPARγ1, PPARγ2, and PPARδ.

7. The method of claim 1 where the nuclear receptor or ligand binding domain thereof comprises a ligand binding domain selected from the group consisting of amino acids 143-462 of human RARα, amino acids 122-410 of rat T3Rα1, amino acids 227-463 of mouse RXRγ, and amino acids 251-595 of human ER.

8. The method of claim 1 where CBP, p300, or other nuclear receptor co-activator, or a binding portion thereof is selected from the group consisting of full-length human CBP, full-length mouse CBP, amino acid residues 1-113 of human CBP, and amino acid residues 1-453 of human CBP.

9. The method of claim 1 where the first fluorescent reagent is selected from the group consisting of XL665 and Europium cryptate (Eu3+K).

10. The method of claim 1 where the second fluorescent reagent is selected from the group consisting of XL665 and Europium cryptate (Eu3+K).

11. A method of identifying an agonist of a nuclear receptor that comprises providing:

(a) a nuclear receptor or ligand binding domain thereof labeled with a first fluorescent reagent;

(b) a binding portion of a nuclear receptor co-activator, where the binding portion contains the amino acid sequence LXXLL, and where the binding portion is labeled with a second fluorescent reagent; and

(c) a substance suspected of being an agonist of the nuclear receptor;

under conditions such that, if the substance is an agonist of the nuclear receptor, binding between the nuclear receptor or ligand binding domain thereof and the binding portion of the nuclear receptor co-activator will take place; and

(d) measuring fluorescence resonance energy transfer (FRET) between the first and second fluorescent reagents;

where the occurrence of FRET indicates that the substance is an agonist of the nuclear receptor.

12. The method of claim 11 where the binding portion of a nuclear receptor co-activator is selected from the group consisting of human RIP-140, human SRC-1, mouse TIF-2, human or mouse CBP, human or mouse p300, mouse TIF-1, and human TRIP proteins.

13. A method of identifying an agonist of a nuclear receptor that comprises providing:

(a) a nuclear receptor or ligand binding domain thereof labeled with a first fluorescent reagent;

(b) a polypeptide containing the amino acid sequence LXXLL where the polypeptide is labeled with a second fluorescent reagent; and

(c) a substance suspected of being an agonist of the nuclear receptor;

under conditions such that, if the substance is an agonist of the nuclear receptor, binding between the nuclear receptor or ligand binding domain thereof and the polypeptide will take place; and

(d) measuring fluorescent resonance energy transfer (FRET) between the first and second fluorescent reagents;

where the occurrence of FRET indicates that the substance is an agonist of the nuclear receptor.

14. A method for identifying an antagonist of a nuclear receptor that comprises providing:

(a) a nuclear receptor or ligand binding domain thereof labeled with a first fluorescent reagent;

(b) CBP, p300, or other nuclear receptor co-activator, or a binding portion thereof, labeled with a second fluorescent reagent;

(c) an agonist of the nuclear receptor; and

(d) a substance suspected of being an antagonist of the nuclear receptor;

under conditions such that, in the absence of the substance, binding between the nuclear receptor or ligand binding domain thereof and CBP, p300, or other nuclear receptor co-activator, or a binding portion thereof will occur; and

(e) measuring fluorescence resonance energy transfer (FRET) between the first and second fluorescent reagents when the substance is present and measuring FRET between the first and second fluorescent reagents when the substance is absent;

where the a decrease in FRET when the substance is present indicates that the substance is an antagonist of the nuclear receptor.

15. The method of claim 14 where the nuclear receptor or ligand binding domain thereof is selected from the group consisting of steroid receptors, thyroid hormone receptors, retinoic acid receptors, peroxisome proliferator-activated receptors, retinoid X receptors, glucocorticoid receptors, vitamin D receptors, LXR, and FXR.

16. The method of claim 14 where the nuclear receptor or ligand binding domain thereof is selected from the group consisting of a full-length nuclear receptor, a ligand binding domain of a nuclear receptor, and an AF-2 site of a nuclear receptor.

17. The method of claim 14 where the nuclear receptor or ligand binding domain thereof comprises an AF-2 site of a nuclear receptor.

18. The method of claim 14 where the nuclear receptor or ligand binding domain thereof is selected from the group consisting of a

full-length PPAR, a ligand binding domain of a PPAR, and amino acid residues 176-478 of human PPARγ1.

19. The method of claim 14 where the nuclear receptor or ligand binding domain thereof is selected from the group consisting of PPARα, PPARγ1, PPARγ2, and PPARδ.

20. The method of claim 14 where the nuclear receptor or ligand binding domain thereof comprises a ligand binding domain selected from the group consisting of amino acids 143-462 of human RARα, amino acids 122-410 of rat T3Rα1, amino acids 227-463 of mouse RXRγ, and amino acids 251-595 of human ER.

21. The method of claim 14 where CBP, p300, or other nuclear receptor co-activator, or a binding portion thereof is selected from the group consisting of full-length CBP, amino acid residues 1-113 of human CBP, and amino acid residues 1-453 of human CBP.

22. The method of claim 14 where the first fluorescent reagent is selected from the group consisting of XL665 and Europium cryptate (Eu3+K).

23. The method of claim 14 where the second fluorescent reagent is selected from the group consisting of XL665 and Europium cryptate (Eu3+K).

24. A nuclear receptor or ligand binding domain thereof labeled with a fluorescent reagent.

25. The nuclear receptor or ligand binding domain thereof of claim 24 where the nuclear receptor or ligand binding domain thereof is selected from the group consisting of PPARα, PPARγ1, PPARγ2, PPARδ, a ligand binding domain of PPARα, PPARγ1, PPARγ2, or PPARδ, and amino acid residues 176-478 of human PPARγ1 and the fluorescent reagent is selected from the group consisting of XL665 and Europium cryptate (Eu3+K).

26. CBP, p300, or other nuclear receptor co-activator, or a binding portion thereof, labeled with a fluorescent reagent.

27. The CBP, p300, or other nuclear receptor co-activator, or a binding portion thereof, of claim 26 where the fluorescent reagent is selected from the group consisting of XL665 and Europium cryptate (Eu3+K).

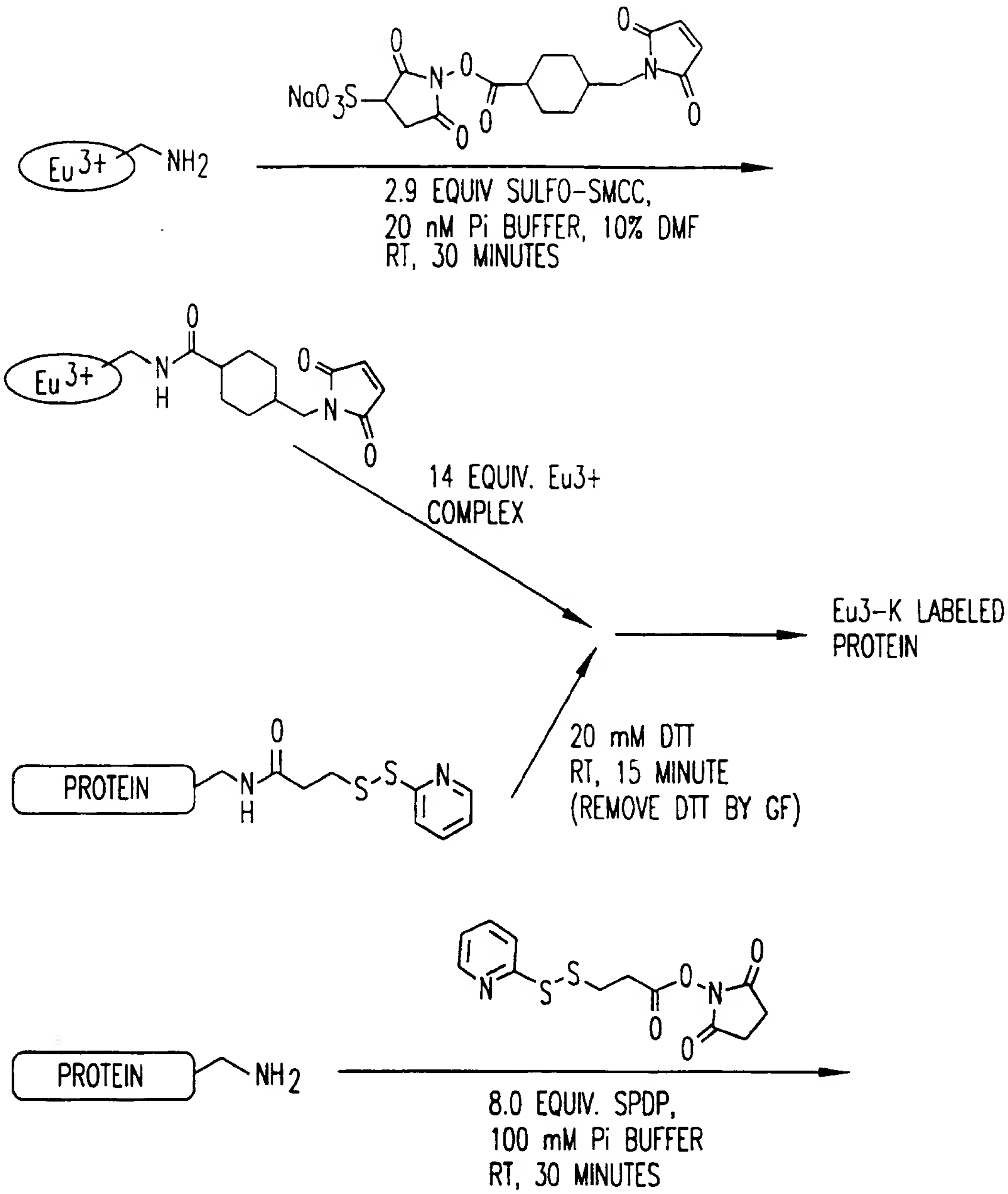

NaO3S
Eu3+
NH2
2.9 EQUIV SULFO-SMCC,
20 nM Pi BUFFER, 10% DMF
RT, 30 MINUTES
14 EQUIV. Eu3+
COMPLEX
Eu3-K LABELED
PROTEIN
PROTEIN
20 mM DTT
RT, 15 MINUTE
(REMOVE DTT BY GF)
PROTEIN
NH2
8.0 EQUIV. SPDP,
100 mM Pi BUFFER
RT, 30 MINUTES

FIG.1

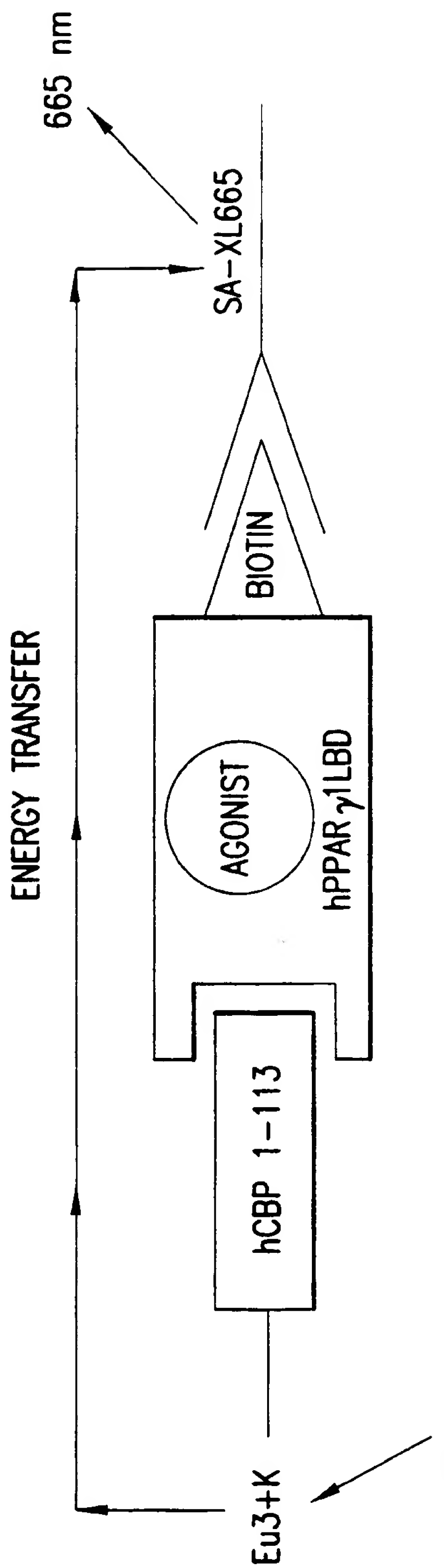

665 nm
SA-XL665
BIOTIN
ENERGY TRANSFER
AGONIST
hPPARγ1LBD
hCBP 1-113
Eu3+K
337 nm

FIG.2

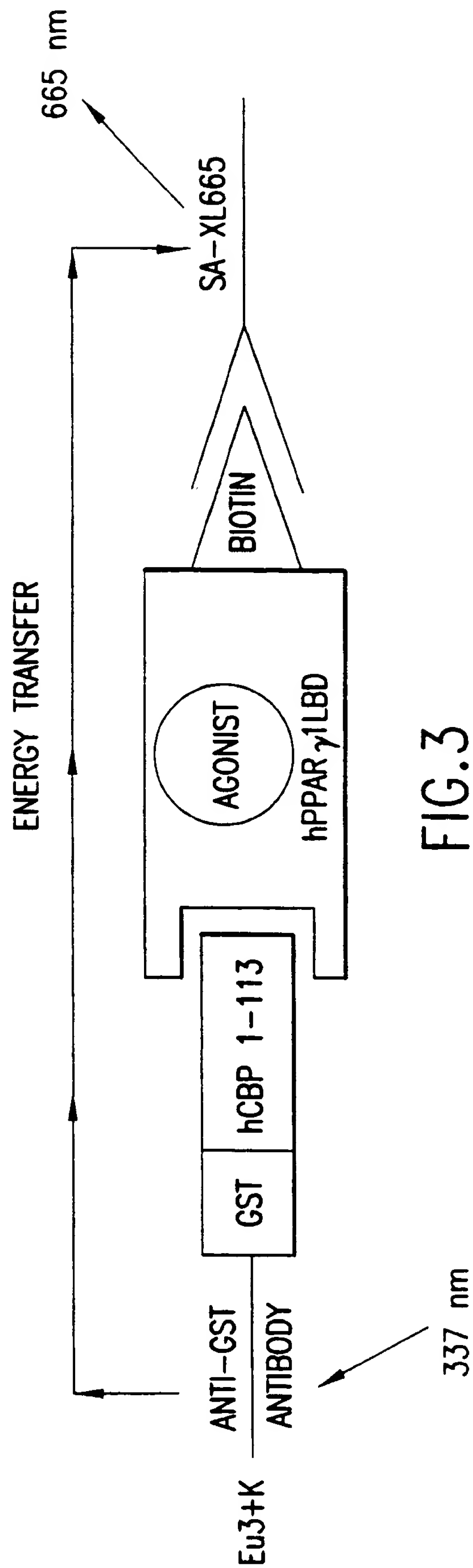

665 nm
SA-XL665
BIOTIN
ENERGY TRANSFER
AGONIST
hPPARγ1LBD
GST
hCBP 1-113
ANTI-GST
ANTIBODY
Eu3+K
337 nm

FIG.3

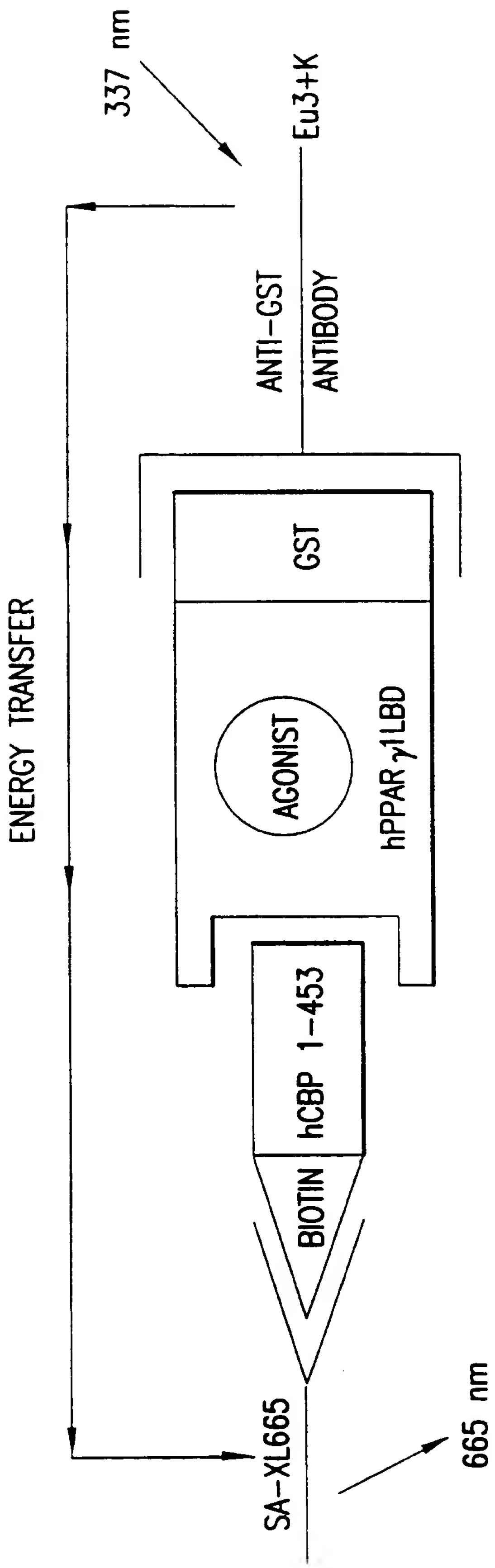

337 nm
Eu3+K
ANTI-GST
ANTIBODY
ENERGY TRANSFER
GST
AGONIST
hPPARγ1LBD
hCBP 1-453
BIOTIN
SA-XL665
665 nm

FIG.4

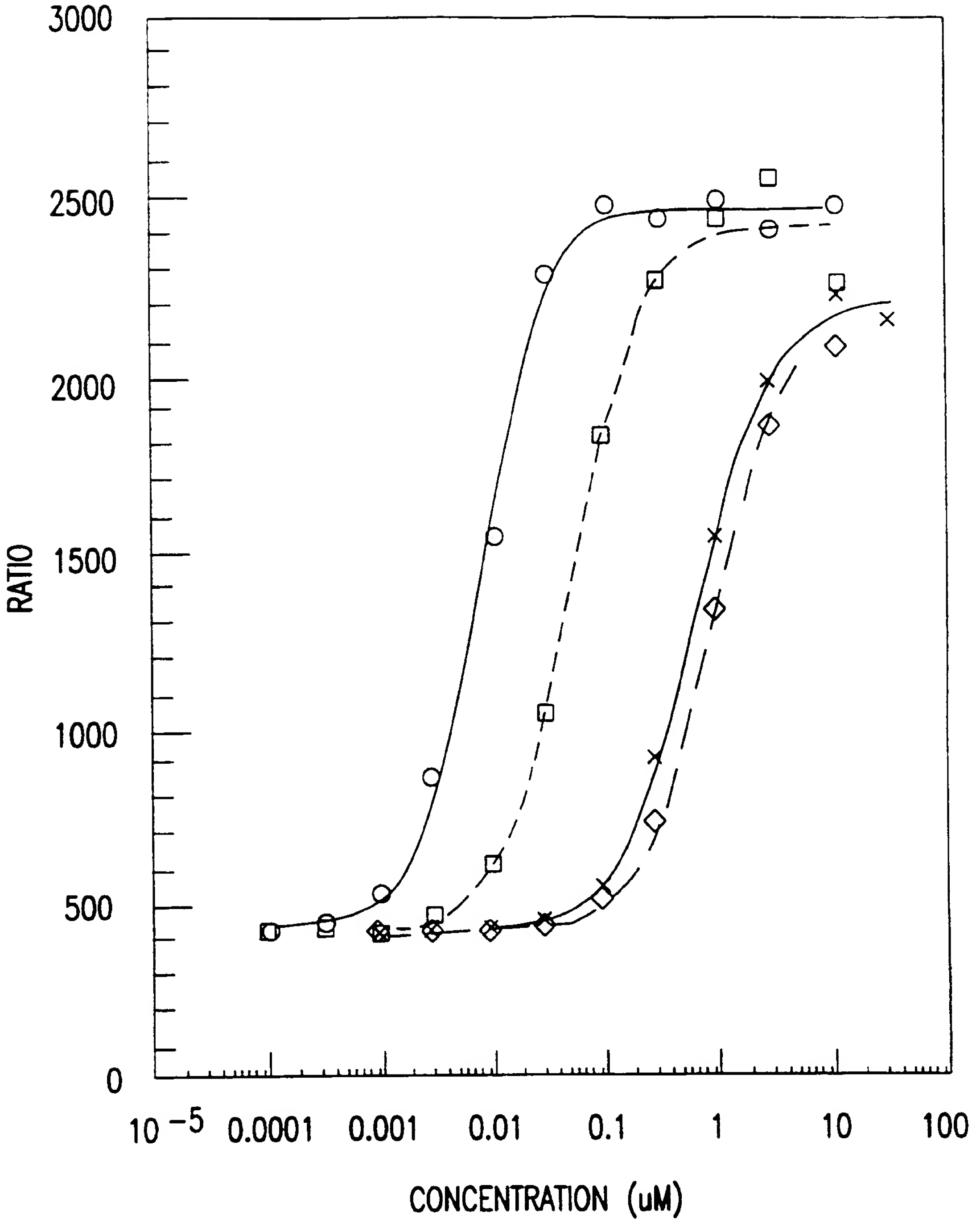

3000
2500
2000
1500
1000
500
0
RATIO
10^{-5}
0.0001
0.001
0.01
0.1
1
10
100
CONCENTRATION (uM)

FIG.5

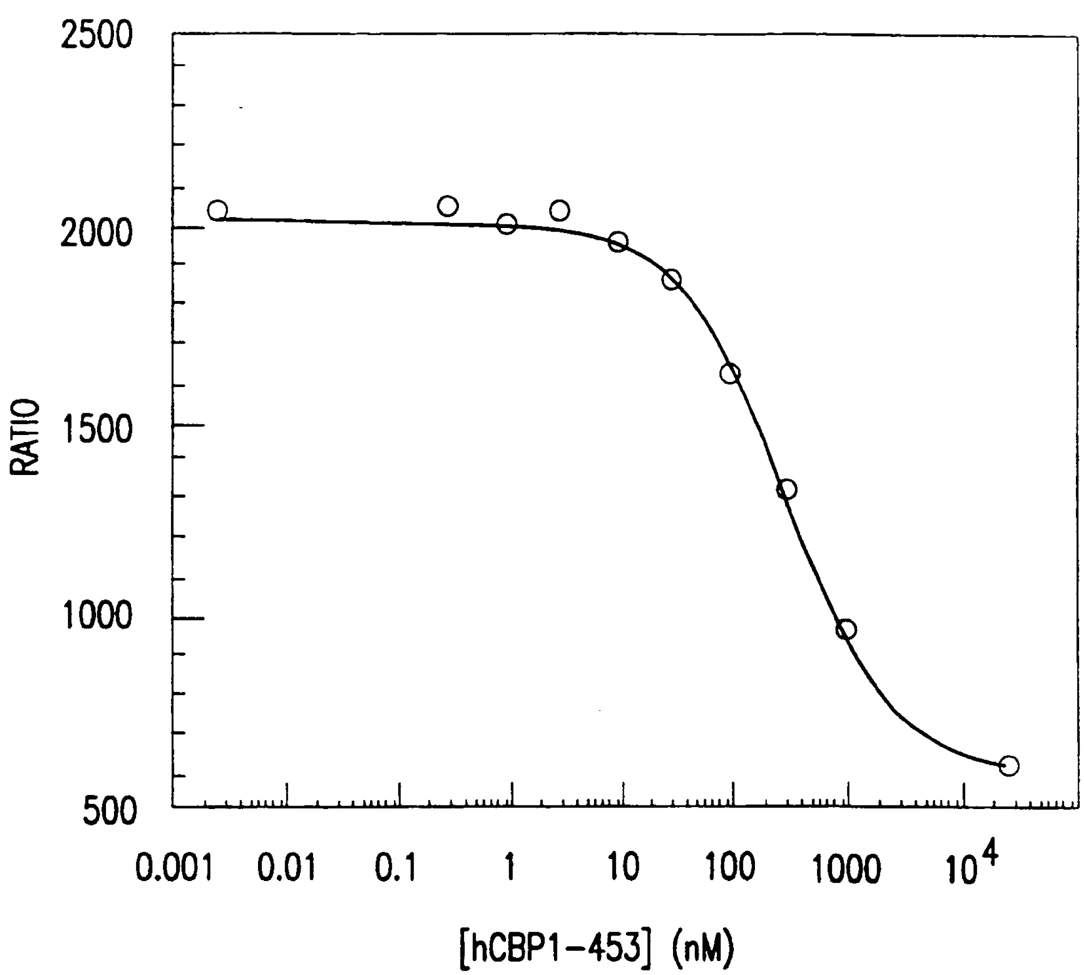

2500
2000
1500
1000
500
RATIO
0.001
0.01
0.1
1
10
100
1000
10^4
[hCBP1-453] (nM)

FIG.6

1 MAENLLDGPPNPKRAKLSSPGFSANDSTDFGSLFDLENDLPDELIPNGGELGLLNSGNLV
61 PDAASKHKQLSELLRGGSGSSINPGIGNVSASSPVQQGLGGQAQGQPNSANMASLSAMGK
121 SPLSQGDSSAPSLPKQAASTSGPTPAASQALNPQAQKQVGLATSSPATSQTGPGICMNAN
181 FNQTHPGLLNSNSGHSLINQASQGQAQVMNGSLGAAGRGRGAGMPYPTPAMQGASSSVLA
241 ETLTQVSPQMTGHAGLNTAQAGGMAKMGITGNTSPFGQPFSQAGGQPMGATGVNPQLASK
301 QSMVNSLPTFPTDIKNTSVTNVPNMSQMQTSVGIVPTQAIATGPTADPEKRKLIQQQLVL
361 LLHAHKCQRREQANGEVRACSLPHCRTMKNVLNHMTHCQAGKACQ

FIG.7A

1 cgagccccga cccccgtccg ggccctcgcc ggccgcgccg cccgtgcccg gggctgtttt
61 cccgagcagg tgaaaatggc tgagaacttg ctggacggac cgcccaaccc caaaagagcc
121 aaactcagct cgcccggttt ctcggcgaat gacagcacag attttggatc attgtttgac
181 ttggaaaatg atcttcctga tgagctgata cccaatggag gagaattagg ccttttaaac
241 agtgggaacc ttgttccaga tgctgcttcc aaacataaac aactgtcgga gcttctacga
301 ggaggcagcg gctctagtat caacccagga ataggaaatg tgagcgccag cagccccgtg
361 cagcagggcc tgggtggcca ggctcaaggg cagccgaaca gtgctaacat ggccagcctc
421 agtgccatgg gcaagagccc tctgagccag ggagattctt cagcccccag cctgcctaaa
481 caggcagcca gcacctctgg gcccaccccc gctgcctccc aagcactgaa tccgcaagca
541 caaaagcaag tggggctggc gactagcagc cctgccacgt cacagactgg acctggtatc
601 tgcatgaatg ctaactttaa ccagacccac ccaggcctcc tcaatagtaa ctctggccat
661 agcttaatta atcaggcttc acaagggcag gcgcaagtca tgaatggatc tcttggggct
721 gctggcagag gaaggggagc tggaatgccg taccctactc cagccatgca gggcgcctcg
781 agcagcgtgc tggctgagac cctaacgcag gtttccccgc aaatgactgg tcacgcggga
841 ctgaacaccg cacaggcagg aggcatggcc aagatgggaa taactgggaa cacaagtcca
901 tttggacagc cctttagtca agctggaggg cagccaatgg gagccactgg agtgaacccc
961 cagttagcca gcaaacagag catggtcaac agtttgccca ccttccctac agatatcaag
1021 aatacttcag tcaccaacgt gccaaatatg tctcagatgc aaacatcagt gggaattgta
1081 cccacacaag caattgcaac aggccccact gcagatcctg aaaaacgcaa actgatacag
1141 cagcagctgg ttctactgct tcatgctcat aagtgtcaga gacgagagca agcaaacgga
1201 gaggttcggg cctgctcgct cccgcattgt cgaaccatga aaaacgtttt gaatcacatg
1261 acgcattgtc aggctgggaa agcctgccaa

FIG.7B

1 MVDTESPLCPLSPLEAGDLESPLSEEFLQEMGNIQEISQSIGEDSSGSFGFTEYQYLGSC
61 PGSDGSVITDTLSPASSPSSVTYPVVPGSVDESPSGALNIECRICGDKASGYHYGVHACE
121 GCKGFFRRTIRLKLVYDKCDRSCKIQKKNRNKCQYCRFHKCLSVGMSHNAIRFGRMPRSE
181 KAKLKAEILTCEHDIEDSETADLKSLAKRIYEAYLKNFNMNKVKARVILSGKASNNPPFV
241 IHDMETLCMAEKTLVAKLVANGIQNKEVEVRIFHCCQCTSVETVTELTEFAKAIPAFANL
301 DLNDQVTLLKYGVYEAIFAMLSSVMNKDGMLVAYGNGFITREFLKSLRKPFCDIMEPKFD
361 FAMKFNALELDDSDISLFVAAIICCGDRPGLLNVGHIEKMQEGIVHVLRLHLQSNHPDDI
421 FLPKLLQKMADLRQLVTEHAQLVQIIKKTESDAALHPLLQEIYRDMY

FIG.8A

1 ggcccaggct gaagctcagg gccctgtctg ctctgtggac tcaacagttt gtggcaagac
61 aagctcagaa ctgagaagct gtcaccacag ttctggaggc tgggaagttc aagatcaaag
121 tgccagcaga ttcagtgtca tgtgaggacg tgcttcctgc ttcatagata agagtagctt
181 ggagctcggc ggcacaacca gcaccatctg gtcgcgatgg tggacacgga aagcccactc
241 tgcccccctct ccccactcga ggccggcgat ctagagagcc cgttatctga agagttcctg
301 caagaaatgg gaaacatcca agagatttcg caatccatcg gcgaggatag ttctggaagc
361 tttggcttta cggaatacca gtatttagga agctgtcctg gctcagatgg ctcggtcatc
421 acggacacgc tttcaccagc ttcgagcccc tcctcggtga cttatcctgt ggtccccggc
481 agcgtggacg agtctcccag tggagcattg aacatcgaat gtagaatctg cggggacaag
541 gcctcaggct atcattacgg agtccacgcg tgtgaaggct gcaagggctt ctttcggcga
601 acgattcgac tcaagctggt gtatgacaag tgcgaccgca gctgcaagat ccagaaaaag
661 aacagaaaca aatgccagta ttgtcgattt cacaagtgcc tttctgtcgg gatgtcacac
721 aacgcgattc gttttggacg aatgccaaga tctgagaaag caaaactgaa agcagaaatt
781 cttacctgtg aacatgacat agaagattct gaaactgcag atctcaaatc tctggccaag
841 agaatctacg aggcctactt gaagaacttc aacatgaaca aggtcaaagc ccgggtcatc
901 ctctcaggaa aggccagtaa caatccacct tttgtcatac atgatatgga gacactgtgt
961 atggctgaga agacgctggt ggccaagctg gtggccaatg gcatccagaa caaggaggtg
1021 gaggtccgca tctttcactg ctgccagtgc acgtcagtgg agaccgtcac ggagctcacg
1081 gaattcgcca aggccatccc agcgttcgca aacttggacc tgaacgatca agtgacattg
1141 ctaaaatacg gagtttatga ggccatattc gccatgctgt cttctgtgat gaacaaagac
1201 gggatgctgg tagcgtatgg aaatgggttt ataactcgtg aattcctaaa aagcctaagg
1261 aaaccgttct gtgatatcat ggaacccaag tttgattttg ccatgaagtt caatgcactg
1321 gaactggatg acagtgatat ctcccttttt gtggctgcta tcatttgctg tggagatcgt
1381 cctggccttc taaacgtagg acacattgaa aaaatgcagg agggtattgt acatgtgctc
1441 agactccacc tgcagagcaa ccacccggac gatatctttc tcttcccaaa acttcttcaa
1501 aaaatggcag acctccggca gctggtgacg gagcatgcgc agctggtgca gatcatcaag
1561 aagacggagt cggatgctgc gctgcacccg ctactgcagg agatctacag ggacatgtac
1621 tgagttcctt cagatcagcc acaccttttc caggagttct gaagctgaca gcactacaaa
1681 ggagacgggg gagcagcacg attttgcaca aatatccacc actttaacct tagagcttgg
1741 acagtctgag ctgtaggtaa ccggcatatt attccatatc tttgttttaa ccagtacttc
1801 taagagcata gaactcaaat gctgggggag gtggctaatc tcaggactgg gaag

FIG.8B

1 MTMVDTEIAFWPTNFGISSVDLSVMEDHSHSFDIKPFTTVDFSSISTPHYEDIPFTRTDP
61 VVADYKYDLKLQEYQSAIKVEPASPPYYSEKTQLYNKPHEEPSNSLMAIECRVCGDKASG
121 FHYGVHACEGCKGFFRRTIRLKLIYDRCDLNCRIHKKSRNKCQYCRFQKCLAVGMSHNAI
181 RFGRIAQAEKEKLLAEISSDIDQLNPESADLRQALAKHLYDSYIKSFPLTKAKARAILTG
241 KTTDKSPFVIYDMNSLMMGEDKIKFKHITPLQEQSKEVAIRIFQGCQFRSVEAVQEITEY
301 AKSIPGFVNLDLNDQVTLLKYGVHEIIYTMLASLMNKDGVLISEGQGFMTREFLKSLRKP
361 FGDFMEPKFEFAVKFNALELDDSDLAIFIAVIILSGDRPGLLNVKPIEDIQDNLLQALEL
421 QLKLNHPESSQUAKLLQKMTDLRQIVTEHVQLLQVIKKTETDMSLHPLLQEIYKDLY

FIG.9A

1 ccgaccttac cccaggcggc cttgacgttg gtcttgtcgg caggagacag caccatggtg
61 ggttctctct gagtctggga attcccgagc ccgagccgca gccgccgcct gggggcttg
121 ggtcggcctc gaggacaccg gagaggggcg ccacgccgcc gtggccgcag aaatgaccat
181 ggttgacaca gagatcgcat tctggcccac caactttggg atcagctccg tggatctctc
241 cgtaatggaa gaccactccc actcctttga tatcaagccc ttcactactg ttgacttctc
301 cagcatttct actccacatt acgaagacat tccattcaca agaacagatc cagtggttgc
361 agattacaag tatgacctga aacttcaaga gtaccaaagt gcaatcaaag tggagcctgc
421 atctccacct tattattctg agaagactca gctctacaat aagcctcatg aagagccttc
481 caactccctc atggcaattg aatgtcgtgt ctgtggagat aaagcttctg gatttcacta
541 tggagttcat gcttgtgaag gatgcaaggg tttcttccgg agaacaatca gattgaagct
601 tatctatgac agatgtgatc ttaactgtcg gatccacaaa aaaagtagaa ataaatgtca
661 gtactgtcgg tttcagaaat gccttgcagt ggggatgtct cataatgcca tcaggtttgg
721 gcggatcgca caggccgaga aggagaagct gttggcggag atctccagtg atatcgacca
781 gctgaatcca gagtccgctg acctccgtca ggccctggca aaacatttgt atgactcata
841 cataaagtcc ttcccgctga ccaaagcaaa ggcgagggcg atcttgacag gaaagacaac
901 agacaaatca ccattcgtta tctatgacat gaattcctta atgatgggag aagataaaat
961 caagttcaaa cacatcaccc ccctgcagga gcagagcaaa gaggtggcca tccgcatctt
1021 tcagggctgc cagtttcgct ccgtggaggc tgtgcaggag atcacagagt atgccaaaag
1081 cattcctggt tttgtaaatc ttgacttgaa cgaccaagta actctcctca aatatggagt
1141 ccacgagatc atttacacaa tgctggcctc cttgatgaat aaagatgggg ttctcatatc
1201 cgagggccaa ggcttcatga caagggagtt tctaaagagc ctgcgaaagc cttttggtga
1261 ctttatggag cccaagtttg agtttgctgt gaagttcaat gcactggaat tagatgacag
1321 cgacttggca atatttattg ctgtcattat tctcagtgga gaccgcccag gtttgctgaa
1381 tgtgaagccc attgaagaca ttcaagacaa cctgctacaa gccctggagc tccagctgaa
1441 gctgaaccac cctgagtcct cacagctgtt tgccaagctg ctccagaaaa tgacagacct
1501 cagacagatt gtcacggaac acgtgcagct actgcaggtg atcaagaaga cggagacaga
1561 catgagtctt cacccgctcc tgcaggagat ctacaaggac ttgtactagc agagagtcct
1621 gagccactgc caacatttcc cttcttccag ttgcactatt ctgagggaaa atctgaccat
1681 aagaaattta ctgtgaaaaa gcgttttaaa aagaaaaggg tttagaatat gatctatttt
1741 atgcatattg tttataaaga cacatttaca atttactttt aatattaaaa attaccatat
1801 tatgaaattg c

FIG.9B

1 MEQPQEEAPEVREEEEKEEVAEAEGAPELNGGPQHALPSSSYTDLSRSSSPPSLLDQLQM
61 GCPGASCGSLNMECRVCGDKASGFHYGVHACEGCKGFFRRTIRMKLEYEKCERSCKIQKK
121 NRNKCQYCRFQKCLALGMSHNAIRFGRMPEAEKRKLVAGLTANEGSQYNPQVADLKAFSK
181 HIYNAYLKNFNMTKKKARSILTGKASHTAPFVIHDIETLWQAEKGLVWKQLVNGLPPYKE
241 ISVHVFYRCQCTTVETVRELTEFAKSIPSFSSLFLNDQVTLLKYGVHEAIFAMLASIVNK
301 DGLLVANGSGFVTREFLRSLRKPFSDIIEPKFEFAVKFNALELDDSDLALFIAAIILCGD
361 RPGLMNVPRVEAIQDTILRALEFHLQANHPDAQYLFPKLLQKMADLRQLVTEHAQMMQRI
421 KKTETETSLHPLLQEIYKDMY

FIG.10A

```
1    gaattctgcg gagcctgcgg gacggcggcg ggttggcccg taggcagccg ggacagtgtt
61   gtacagtgtt ttgggcatgc acgtgatact cacacagtgg cttctgctca ccaacagatg
121  aagacagatg caccaacgag ggtctggaat ggtctggagt ggtctggaaa gcagggtcag
181  ataccccctgg aaaactgaag cccgtggagc aatgatctct acaggactgc ttcaaggctg
241  atgggaacca ccctgtagag gtccatctgc gttcagaccc agacgatgcc agagctatga
301  ctgggcctgc aggtgtggcg ccgaggggag atcagccatg gagcagccac aggaggaagc
361  ccctgaggtc cgggaagagg aggagaaaga ggaagtggca gaggcagaag gagcccccaga
421  gctcaatggg ggaccacagc atgcacttcc ttccagcagc tacacagacc tctcccggag
481  ctcctcgcca ccctcactgc tggaccaact gcagatgggc tgtgacgggg cctcatgcgg
541  cagcctcaac atggagtgcc gggtgtgcgg ggacaaggca tcgggcttcc actacggtgt
601  tcatgcatgt gaggggtgca agggcttctt ccgtcgtacg atccgcatga agctggagta
661  cgagaagtgt gagcgcagct gcaagattca gaagaagaac cgcaacaagt gccagtactg
721  ccgcttccag aagtgcctgg cactgggcat gtcacacaac gctatccgtt ttggtcggat
781  gccggaggct gagaagagga agctggtggc agggctgact gcaaacgagg ggagccagta
841  caacccacag gtggccgacc tgaaggcctt ctccaagcac atctacaatg cctacctgaa
901  aaacttcaac atgaccaaaa agaaggcccg cagcatcctc accggcaaag ccagccacac
961  ggcgcccttt gtgatccacg acatcgagac attgtggcag gcagagaagg ggctggtgtg
1021 gaagcagttg gtgaatggcc tgcctcccta caaggagatc agcgtgcacg tcttctaccg
1081 ctgccagtgc accacagtgg agaccgtgcg ggagctcact gagttcgcca agagcatccc
1141 cagcttcagc agcctcttcc tcaacgacca ggttaccctt ctcaagtatg gcgtgcacga
1201 ggccatcttc gccatgctgg cctctatcgt caacaaggac gggctgctgg tagccaacgg
1261 cagtggcttt gtcacccgtg agttcctgcg cagcctccgc aaacccttca gtgatatcat
1321 tgagcctaag tttgaatttg ctgtcaagtt caacgccctg gaacttgatg acagtgacct
1381 ggccctattc attgcggcca tcattctgtg tggagaccgg ccaggcctca tgaacgttcc
1441 acgggtggag gctatccagg acaccatcct gcgtgccctc gaattccacc tgcaggccaa
1501 ccaccctgat gcccagtacc tcttccccaa gctgctgcag aagatggctg acctgcggca
1561 actggtcacc gagcacgccc agatgatgca gcggatcaag aagaccgaaa ccpagacctc
1621 gctgcacccct ctgctccagg agatctacaa ggacatgtac taacggcggc acccaggcct
1681 ccctgcagac tccaatgggg ccagcactgg aggggcccac ccacatgact tttccattga
1741 ccagctctct tcctgtcttt gttgtctccc tctttctcag ttcctctttc ttttctaatt
1801 cctgttgctc tgtttcttcc tttctgtagg tttctctctt cccttctccc ttctcccttg
1861 ccctcccttt ctctctccta tccccacgtc tgtcctcctt tcttattctg tgagatgttt
1921 tgtattattt caccagcagc atagaacagg acctctgctt ttgcacacct tttccccagg
1981 agcagaagag agtgggcctg ccctctgccc catcattgca cctgcaggct taggtcctca
2041 cttctgtctc ctgtcttcag agcaaaagac ttgagccatc caaagaaaca ctaagctctc
2101 tgggcctggg ttccagggaa ggctaagcat ggcctggact gactgcagcc ccctatagtc
2161 atggggtccc tgctgcaaag gacagtggca gaccccggca gtagagccga gatgcctccc
2221 caagactgtc attgcccctc cgatcgtgag gccacccact gacccaatga tcctctccag
2281 cagcacacct cagccccact gacacccagt gtccttccat cttcacactg gtttgccagg
2341 ccaatgttgc tgatggcccc tccagcacac acacataagc actgaaatca ctttacctgc
2401 aggcaccatg cacctccctt ccctccctga ggcaggtgag aacccagaga gaggggcctg
```

FIG.10B

```
2461 caggtgagca ggcagggctg ggccaggtct ccggggaggc aggggtcctg caggtcctgg
2521 tgggtcagcc cagcacctcg cccagtggga gcttcccggg ataaactgag cctgttcatt
2581 ctgatgtcca tttgtcccaa tagctctact gccctcccct tcccctttac tcagcccagc
2641 tggccaccta gaagtctccc tgcacagcct ctagtgtccg gggaccttgt gggaccagtc
2701 ccacaccgct ggtccctgcc ctcccctgct cccaggttga ggtgcgctca cctcagagca
2761 gggccaaagc acagctgggc atgccatgtc tgagcggcgc agagccctcc aggcctgcag
2821 gggcaagggg ctggctggag tctcagagca cagaggtagg agaactgggg ttcaagccca
2881 ggcttcctgg gtcctgcctg gtcctccctc ccaaggagcc attctatgtg actctgggtg
2941 gaagtgccca gcccctgcct gacggnnnnn nngatcactc tctgctggca ggattcttcc
3001 cgctccccac ctacccagct gatgggggtt ggggtgcttc tttcagccaa ggctatgaag
3061 ggacagctgc tgggacccac ctccccccctt ccccggccac atgccgcgtc cctgcccccca
3121 cccgggtctg gtgctgagga tacagctctt ctcagtgtct gaacaatctc caaaattgaa
3181 atgtatattt ttgctaggag ccccagcttc ctgtgttttt aatataaata gtgtacacag
3241 actgacgaaa ctttaaataa atgggaatta aatatttaaa aaaaaaagcg gccgcgaatt
3301 c
```

FIG.10C

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Merck & Co., Inc.

(ii) TITLE OF INVENTION: ASSAYS FOR NUCLEAR RECEPTOR AGONISTS AND ANTAGONISTS USING FLUORESCENCE RESONANCE ENERGY TRANSFER

(iii) NUMBER OF SEQUENCES: 11

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Merck & Co., Inc.
(B) STREET: P.O. Box 2000, 126 E. Lincoln Ave.
(C) CITY: Rahway
(D) STATE: NJ
(E) COUNTRY: USA
(F) ZIP: 07065-0900

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: Windows
(D) SOFTWARE: FastSEQ for Windows Version 2.0b

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Coppola, Joseph A
(B) REGISTRATION NUMBER: 38,413
(C) REFERENCE/DOCKET NUMBER: 20017PCT

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 732-594-6734
(B) TELEFAX: 732-594-4720
(C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 405 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
Met Ala Glu Asn Leu Leu Asp Gly Pro Pro Asn Pro Lys Arg Ala Lys
 1               5                  10                  15
Leu Ser Ser Pro Gly Phe Ser Ala Asn Asp Ser Thr Asp Phe Gly Ser
            20                  25                  30
Leu Phe Asp Leu Glu Asn Asp Leu Pro Asp Glu Leu Ile Pro Asn Gly
        35                  40                  45
Gly Glu Leu Gly Leu Leu Asn Ser Gly Asn Leu Val Pro Asp Ala Ala
    50                  55                  60
Ser Lys His Lys Gln Leu Ser Glu Leu Leu Arg Gly Gly Ser Gly Ser
65                  70                  75                  80
Ser Ile Asn Pro Gly Ile Gly Asn Val Ser Ala Ser Ser Pro Val Gln
                85                  90                  95
Gln Gly Leu Gly Gly Gln Ala Gln Gly Gln Pro Asn Ser Ala Asn Met
            100                 105                 110
Ala Ser Leu Ser Ala Met Gly Lys Ser Pro Leu Ser Gln Gly Asp Ser
        115                 120                 125
Ser Ala Pro Ser Leu Pro Lys Gln Ala Ala Ser Thr Ser Gly Pro Thr
    130                 135                 140
Pro Ala Ala Ser Gln Ala Leu Asn Pro Gln Ala Gln Lys Gln Val Gly
145                 150                 155                 160
Leu Ala Thr Ser Ser Pro Ala Thr Ser Gln Thr Gly Pro Gly Ile Cys
                165                 170                 175
Met Asn Ala Asn Phe Asn Gln Thr His Pro Gly Leu Leu Asn Ser Asn
            180                 185                 190
Ser Gly His Ser Leu Ile Asn Gln Ala Ser Gln Gly Gln Ala Gln Val
        195                 200                 205
Met Asn Gly Ser Leu Gly Ala Ala Gly Arg Gly Arg Gly Ala Gly Met
    210                 215                 220
Pro Tyr Pro Thr Pro Ala Met Gln Gly Ala Ser Ser Ser Val Leu Ala
225                 230                 235                 240
Glu Thr Leu Thr Gln Val Ser Pro Gln Met Thr Gly His Ala Gly Leu
                245                 250                 255
Asn Thr Ala Gln Ala Gly Gly Met Ala Lys Met Gly Ile Thr Gly Asn
            260                 265                 270
Thr Ser Pro Phe Gly Gln Pro Phe Ser Gln Ala Gly Gly Gln Pro Met
        275                 280                 285
Gly Ala Thr Gly Val Asn Pro Gln Leu Ala Ser Lys Gln Ser Met Val
    290                 295                 300
Asn Ser Leu Pro Thr Phe Pro Thr Asp Ile Lys Asn Thr Ser Val Thr
305                 310                 315                 320
Asn Val Pro Asn Met Ser Gln Met Gln Thr Ser Val Gly Ile Val Pro
                325                 330                 335
Thr Gln Ala Ile Ala Thr Gly Pro Thr Ala Asp Pro Glu Lys Arg Lys
            340                 345                 350
Leu Ile Gln Gln Gln Leu Val Leu Leu Leu His Ala His Lys Cys Gln
        355                 360                 365
Arg Arg Glu Gln Ala Asn Gly Glu Val Arg Ala Cys Ser Leu Pro His
    370                 375                 380
Cys Arg Thr Met Lys Asn Val Leu Asn His Met Thr His Cys Gln Ala
385                 390                 395                 400
Gly Lys Ala Cys Gln
                405
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1290 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
CGAGCCCCGA CCCCCGTCCG GGCCCTCGCC GGCCGCGCCG CCCGTGCCCG GGGCTGTTTT     60
CCCGAGCAGG TGAAAATGGC TGAGAACTTG CTGGACGGAC CGCCCAACCC CAAAAGAGCC    120
AAACTCAGCT CGCCCGGTTT CTCGGCGAAT GACAGCACAG ATTTTGGATC ATTGTTTGAC    180
TTGGAAAATG ATCTTCCTGA TGAGCTGATA CCCAATGGAG GAGAATTAGG CCTTTTAAAC    240
AGTGGGAACC TTGTTCCAGA TGCTGCTTCC AAACATAAAC AACTGTCGGA GCTTCTACGA    300
GGAGGCAGCG GCTCTAGTAT CAACCCAGGA ATAGGAAATG TGAGCGCCAG CAGCCCCGTG    360
CAGCAGGGCC TGGGTGGCCA GGCTCAAGGG CAGCCGAACA GTGCTAACAT GGCCAGCCTC    420
AGTGCCATGG GCAAGAGCCC TCTGAGCCAG GGAGATTCTT CAGCCCCCAG CCTGCCTAAA    480
CAGGCAGCCA GCACCTCTGG GCCCACCCCC GCTGCCTCCC AAGCACTGAA TCCGCAAGCA    540
CAAAAGCAAG TGGGGCTGGC GACTAGCAGC CCTGCCACGT CACAGACTGG ACCTGGTATC    600
TGCATGAATG CTAACTTTAA CCAGACCCAC CCAGGCCTCC TCAATAGTAA CTCTGGCCAT    660
AGCTTAATTA ATCAGGCTTC ACAAGGGCAG GCGCAAGTCA TGAATGGATC TCTTGGGGCT    720
GCTGGCAGAG GAAGGGGAGC TGGAATGCCG TACCCTACTC CAGCCATGCA GGGCGCCTCG    780
AGCAGCGTGC TGGCTGAGAC CCTAACGCAG GTTTCCCCGC AAATGACTGG TCACGCGGGA    840
CTGAACACCG CACAGGCAGG AGGCATGGCC AAGATGGGAA TAACTGGGAA CACAAGTCCA    900
TTTGGACAGC CCTTTAGTCA AGCTGGAGGG CAGCCAATGG GAGCCACTGG AGTGAACCCC    960
CAGTTAGCCA GCAAACAGAG CATGGTCAAC AGTTTGCCCA CCTTCCCTAC AGATATCAAG   1020
AATACTTCAG TCACCAACGT GCCAAATATG TCTCAGATGC AAACATCAGT GGGAATTGTA   1080
CCCACACAAG CAATTGCAAC AGGCCCCACT GCAGATCCTG AAAAACGCAA ACTGATACAG   1140
CAGCAGCTGG TTCTACTGCT TCATGCTCAT AAGTGTCAGA GACGAGAGCA AGCAAACGGA   1200
GAGGTTCGGG CCTGCTCGCT CCCGCATTGT CGAACCATGA AAAACGTTTT GAATCACATG   1260
ACGCATTGTC AGGCTGGGAA AGCCTGCCAA                                    1290
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 468 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Met Val Asp Thr Glu Ser Pro Leu Cys Pro Leu Ser Pro Leu Glu Ala
 1               5                  10                  15
Gly Asp Leu Glu Ser Pro Leu Ser Glu Glu Phe Leu Gln Glu Met Gly
            20                  25                  30
Asn Ile Gln Glu Ile Ser Gln Ser Ile Gly Glu Asp Ser Ser Gly Ser
        35                  40                  45
Phe Gly Phe Thr Glu Tyr Gln Tyr Leu Gly Ser Cys Pro Gly Ser Asp
    50                  55                  60
```

Gly Ser Val Ile Thr Asp Thr Leu Ser Pro Ala Ser Ser Pro Ser Ser
65 70 75 80
Val Thr Tyr Pro Val Val Pro Gly Ser Val Asp Glu Ser Pro Ser Gly
85 90 95
Ala Leu Asn Ile Glu Cys Arg Ile Cys Gly Asp Lys Ala Ser Gly Tyr
100 105 110
His Tyr Gly Val His Ala Cys Glu Gly Cys Lys Gly Phe Phe Arg Arg
115 120 125
Thr Ile Arg Leu Lys Leu Val Tyr Asp Lys Cys Asp Arg Ser Cys Lys
130 135 140
Ile Gln Lys Lys Asn Arg Asn Lys Cys Gln Tyr Cys Arg Phe His Lys
145 150 155 160
Cys Leu Ser Val Gly Met Ser His Asn Ala Ile Arg Phe Gly Arg Met
165 170 175
Pro Arg Ser Glu Lys Ala Lys Leu Lys Ala Glu Ile Leu Thr Cys Glu
180 185 190
His Asp Ile Glu Asp Ser Glu Thr Ala Asp Leu Lys Ser Leu Ala Lys
195 200 205
Arg Ile Tyr Glu Ala Tyr Leu Lys Asn Phe Asn Met Asn Lys Val Lys
210 215 220
Ala Arg Val Ile Leu Ser Gly Lys Ala Ser Asn Asn Pro Pro Phe Val
225 230 235 240
Ile His Asp Met Glu Thr Leu Cys Met Ala Glu Lys Thr Leu Val Ala
245 250 255
Lys Leu Val Ala Asn Gly Ile Gln Asn Lys Glu Val Glu Val Arg Ile
260 265 270
Phe His Cys Cys Gln Cys Thr Ser Val Glu Thr Val Thr Glu Leu Thr
275 280 285
Glu Phe Ala Lys Ala Ile Pro Ala Phe Ala Asn Leu Asp Leu Asn Asp
290 295 300
Gln Val Thr Leu Leu Lys Tyr Gly Val Tyr Glu Ala Ile Phe Ala Met
305 310 315 320
Leu Ser Ser Val Met Asn Lys Asp Gly Met Leu Val Ala Tyr Gly Asn
325 330 335
Gly Phe Ile Thr Arg Glu Phe Leu Lys Ser Leu Arg Lys Pro Phe Cys
340 345 350
Asp Ile Met Glu Pro Lys Phe Asp Phe Ala Met Lys Phe Asn Ala Leu
355 360 365
Glu Leu Asp Asp Ser Asp Ile Ser Leu Phe Val Ala Ala Ile Ile Cys
370 375 380
Cys Gly Asp Arg Pro Gly Leu Leu Asn Val Gly His Ile Glu Lys Met
385 390 395 400
Gln Glu Gly Ile Val His Val Leu Arg Leu His Leu Gln Ser Asn His
405 410 415
Pro Asp Asp Ile Phe Leu Phe Pro Lys Leu Leu Gln Lys Met Ala Asp
420 425 430
Leu Arg Gln Leu Val Thr Glu His Ala Gln Leu Val Gln Ile Ile Lys
435 440 445
Lys Thr Glu Ser Asp Ala Ala Leu His Pro Leu Leu Gln Glu Ile Tyr
450 455 460
Arg Asp Met Tyr
465

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1854 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
GGCCCAGGCT GAAGCTCAGG GCCCTGTCTG CTCTGTGGAC TCAACAGTTT GTGGCAAGAC     60
AAGCTCAGAA CTGAGAAGCT GTCACCACAG TTCTGGAGGC TGGGAAGTTC AAGATCAAAG    120
TGCCAGCAGA TTCAGTGTCA TGTGAGGACG TGCTTCCTGC TTCATAGATA AGAGTAGCTT    180
GGAGCTCGGC GGCACAACCA GCACCATCTG GTCGCGATGG TGGACACGGA AAGCCCACTC    240
TGCCCCCTCT CCCCACTCGA GGCCGGCGAT CTAGAGAGCC CGTTATCTGA AGAGTTCCTG    300
CAAGAAATGG GAAACATCCA AGAGATTTCG CAATCCATCG GCGAGGATAG TTCTGGAAGC    360
TTTGGCTTTA CGGAATACCA GTATTTAGGA AGCTGTCCTG GCTCAGATGG CTCGGTCATC    420
ACGGACACGC TTTCACCAGC TTCGAGCCCC TCCTCGGTGA CTTATCCTGT GGTCCCCGGC    480
AGCGTGGACG AGTCTCCCAG TGGAGCATTG AACATCGAAT GTAGAATCTG CGGGGACAAG    540
GCCTCAGGCT ATCATTACGG AGTCCACGCG TGTGAAGGCT GCAAGGGCTT CTTTCGGCGA    600
ACGATTCGAC TCAAGCTGGT GTATGACAAG TGCGACCGCA GCTGCAAGAT CCAGAAAAAG    660
AACAGAAACA AATGCCAGTA TTGTCGATTT CACAAGTGCC TTTCTGTCGG GATGTCACAC    720
AACGCGATTC GTTTTGGACG AATGCCAAGA TCTGAGAAAG CAAAACTGAA AGCAGAAATT    780
CTTACCTGTG AACATGACAT AGAAGATTCT GAAACTGCAG ATCTCAAATC TCTGGCCAAG    840
AGAATCTACG AGGCCTACTT GAAGAACTTC AACATGAACA AGGTCAAAGC CCGGGTCATC    900
CTCTCAGGAA AGGCCAGTAA CAATCCACCT TTTGTCATAC ATGATATGGA GACACTGTGT    960
ATGGCTGAGA AGACGCTGGT GGCCAAGCTG GTGGCCAATG GCATCCAGAA CAAGGAGGTG   1020
GAGGTCCGCA TCTTTCACTG CTGCCAGTGC ACGTCAGTGG AGACCGTCAC GGAGCTCACG   1080
GAATTCGCCA AGGCCATCCC AGCGTTCGCA AACTTGGACC TGAACGATCA AGTGACATTG   1140
CTAAAATACG GAGTTTATGA GGCCATATTC GCCATGCTGT CTTCTGTGAT GAACAAAGAC   1200
GGGATGCTGG TAGCGTATGG AAATGGGTTT ATAACTCGTG AATTCCTAAA AAGCCTAAGG   1260
AAACCGTTCT GTGATATCAT GGAACCCAAG TTTGATTTTG CCATGAAGTT CAATGCACTG   1320
GAACTGGATG ACAGTGATAT CTCCCTTTTT GTGGCTGCTA TCATTTGCTG TGGAGATCGT   1380
CCTGGCCTTC TAAACGTAGG ACACATTGAA AAAATGCAGG AGGGTATTGT ACATGTGCTC   1440
AGACTCCACC TGCAGAGCAA CCACCCGGAC GATATCTTTC TCTTCCCAAA ACTTCTTCAA   1500
AAAATGGCAG ACCTCCGGCA GCTGGTGACG GAGCATGCGC AGCTGGTGCA GATCATCAAG   1560
AAGACGGAGT CGGATGCTGC GCTGCACCCG CTACTGCAGG AGATCTACAG GGACATGTAC   1620
TGAGTTCCTT CAGATCAGCC ACACCTTTTC CAGGAGTTCT GAAGCTGACA GCACTACAAA   1680
GGAGACGGGG GAGCAGCACG ATTTTGCACA AATATCCACC ACTTTAACCT TAGAGCTTGG   1740
ACAGTCTGAG CTGTAGGTAA CCGGCATATT ATTCCATATC TTTGTTTTAA CCAGTACTTC   1800
TAAGAGCATA GAACTCAAAT GCTGGGGGAG GTGGCTAATC TCAGGACTGG GAAG         1854
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 478 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Met Thr Met Val Asp Thr Glu Ile Ala Phe Trp Pro Thr Asn Phe Gly
 1               5                  10                  15
```

Ile Ser Ser Val Asp Leu Ser Val Met Glu Asp His Ser His Ser Phe
20 25 30
Asp Ile Lys Pro Phe Thr Thr Val Asp Phe Ser Ser Ile Ser Thr Pro
35 40 45
His Tyr Glu Asp Ile Pro Phe Thr Arg Thr Asp Pro Val Val Ala Asp
50 55 60
Tyr Lys Tyr Asp Leu Lys Leu Gln Glu Tyr Gln Ser Ala Ile Lys Val
65 70 75 80
Glu Pro Ala Ser Pro Pro Tyr Tyr Ser Glu Lys Thr Gln Leu Tyr Asn
85 90 95
Lys Pro His Glu Glu Pro Ser Asn Ser Leu Met Ala Ile Glu Cys Arg
100 105 110
Val Cys Gly Asp Lys Ala Ser Gly Phe His Tyr Gly Val His Ala Cys
115 120 125
Glu Gly Cys Lys Gly Phe Phe Arg Arg Thr Ile Arg Leu Lys Leu Ile
130 135 140
Tyr Asp Arg Cys Asp Leu Asn Cys Arg Ile His Lys Lys Ser Arg Asn
145 150 155 160
Lys Cys Gln Tyr Cys Arg Phe Gln Lys Cys Leu Ala Val Gly Met Ser
165 170 175
His Asn Ala Ile Arg Phe Gly Arg Ile Ala Gln Ala Glu Lys Glu Lys
180 185 190
Leu Leu Ala Glu Ile Ser Ser Asp Ile Asp Gln Leu Asn Pro Glu Ser
195 200 205
Ala Asp Leu Arg Gln Ala Leu Ala Lys His Leu Tyr Asp Ser Tyr Ile
210 215 220
Lys Ser Phe Pro Leu Thr Lys Ala Lys Ala Arg Ala Ile Leu Thr Gly
225 230 235 240
Lys Thr Thr Asp Lys Ser Pro Phe Val Ile Tyr Asp Met Asn Ser Leu
245 250 255
Met Met Gly Glu Asp Lys Ile Lys Phe Lys His Ile Thr Pro Leu Gln
260 265 270
Glu Gln Ser Lys Glu Val Ala Ile Arg Ile Phe Gln Gly Cys Gln Phe
275 280 285
Arg Ser Val Glu Ala Val Gln Glu Ile Thr Glu Tyr Ala Lys Ser Ile
290 295 300
Pro Gly Phe Val Asn Leu Asp Leu Asn Asp Gln Val Thr Leu Leu Lys
305 310 315 320
Tyr Gly Val His Glu Ile Ile Tyr Thr Met Leu Ala Ser Leu Met Asn
325 330 335
Lys Asp Gly Val Leu Ile Ser Glu Gly Gln Gly Phe Met Thr Arg Glu
340 345 350
Phe Leu Lys Ser Leu Arg Lys Pro Phe Gly Asp Phe Met Glu Pro Lys
355 360 365
Phe Glu Phe Ala Val Lys Phe Asn Ala Leu Glu Leu Asp Asp Ser Asp
370 375 380
Leu Ala Ile Phe Ile Ala Val Ile Ile Leu Ser Gly Asp Arg Pro Gly
385 390 395 400
Leu Leu Asn Val Lys Pro Ile Glu Asp Ile Gln Asp Asn Leu Leu Gln
405 410 415
Ala Leu Glu Leu Gln Leu Lys Leu Asn His Pro Glu Ser Ser Gln Leu
420 425 430
Phe Ala Lys Leu Leu Gln Lys Met Thr Asp Leu Arg Gln Ile Val Thr
435 440 445
Glu His Val Gln Leu Leu Gln Val Ile Lys Lys Thr Glu Thr Asp Met
450 455 460

Ser Leu His Pro Leu Leu Gln Glu Ile Tyr Lys Asp Leu Tyr
465 470 475

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1811 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CCGACCTTAC CCCAGGCGGC CTTGACGTTG GTCTTGTCGG CAGGAGACAG CACCATGGTG 60
GGTTCTCTCT GAGTCTGGGA ATTCCCGAGC CCGAGCCGCA GCCGCCGCCT GGGGGGCTTG 120
GGTCGGCCTC GAGGACACCG GAGAGGGGCG CCACGCCGCC GTGGCCGCAG AAATGACCAT 180
GGTTGACACA GAGATCGCAT TCTGGCCCAC CAACTTTGGG ATCAGCTCCG TGGATCTCTC 240
CGTAATGGAA GACCACTCCC ACTCCTTTGA TATCAAGCCC TTCACTACTG TTGACTTCTC 300
CAGCATTTCT ACTCCACATT ACGAAGACAT TCCATTCACA AGAACAGATC CAGTGGTTGC 360
AGATTACAAG TATGACCTGA AACTTCAAGA GTACCAAAGT GCAATCAAAG TGGAGCCTGC 420
ATCTCCACCT TATTATTCTG AGAAGACTCA GCTCTACAAT AAGCCTCATG AAGAGCCTTC 480
CAACTCCCTC ATGGCAATTG AATGTCGTGT CTGTGGAGAT AAAGCTTCTG GATTTCACTA 540
TGGAGTTCAT GCTTGTGAAG GATGCAAGGG TTTCTTCCGG AGAACAATCA GATTGAAGCT 600
TATCTATGAC AGATGTGATC TTAACTGTCG GATCCACAAA AAAAGTAGAA ATAAATGTCA 660
GTACTGTCGG TTTCAGAAAT GCCTTGCAGT GGGGATGTCT CATAATGCCA TCAGGTTTGG 720
GCGGATCGCA CAGGCCGAGA AGGAGAAGCT GTTGGCGGAG ATCTCCAGTG ATATCGACCA 780
GCTGAATCCA GAGTCCGCTG ACCTCCGTCA GGCCCTGGCA AAACATTTGT ATGACTCATA 840
CATAAAGTCC TTCCCGCTGA CCAAAGCAAA GGCGAGGGCG ATCTTGACAG GAAAGACAAC 900
AGACAAATCA CCATTCGTTA TCTATGACAT GAATTCCTTA ATGATGGGAG AAGATAAAAT 960
CAAGTTCAAA CACATCACCC CCCTGCAGGA GCAGAGCAAA GAGGTGGCCA TCCGCATCTT 1020
TCAGGGCTGC CAGTTTCGCT CCGTGGAGGC TGTGCAGGAG ATCACAGAGT ATGCCAAAAG 1080
CATTCCTGGT TTTGTAAATC TTGACTTGAA CGACCAAGTA ACTCTCCTCA AATATGGAGT 1140
CCACGAGATC ATTTACACAA TGCTGGCCTC CTTGATGAAT AAAGATGGGG TTCTCATATC 1200
CGAGGGCCAA GGCTTCATGA CAAGGGAGTT TCTAAAGAGC CTGCGAAAGC CTTTTGGTGA 1260
CTTTATGGAG CCCAAGTTTG AGTTTGCTGT GAAGTTCAAT GCACTGGAAT TAGATGACAG 1320
CGACTTGGCA ATATTTATTG CTGTCATTAT TCTCAGTGGA GACCGCCCAG GTTTGCTGAA 1380
TGTGAAGCCC ATTGAAGACA TTCAAGACAA CCTGCTACAA GCCCTGGAGC TCCAGCTGAA 1440
GCTGAACCAC CCTGAGTCCT CACAGCTGTT TGCCAAGCTG CTCCAGAAAA TGACAGACCT 1500
CAGACAGATT GTCACGGAAC ACGTGCAGCT ACTGCAGGTG ATCAAGAAGA CGGAGACAGA 1560
CATGAGTCTT CACCCGCTCC TGCAGGAGAT CTACAAGGAC TTGTACTAGC AGAGAGTCCT 1620
GAGCCACTGC CAACATTTCC CTTCTTCCAG TTGCACTATT CTGAGGGAAA ATCTGACCAT 1680
AAGAAATTTA CTGTGAAAAA GCGTTTTAAA AAGAAAAGGG TTTAGAATAT GATCTATTTT 1740
ATGCATATTG TTTATAAAGA CACATTTACA ATTTACTTTT AATATTAAAA ATTACCATAT 1800
TATGAAATTG C 1811

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 441 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Met Glu Gln Pro Gln Glu Glu Ala Pro Glu Val Arg Glu Glu Glu Glu
 1               5                  10                  15
Lys Glu Glu Val Ala Glu Ala Glu Gly Ala Pro Glu Leu Asn Gly Gly
            20                  25                  30
Pro Gln His Ala Leu Pro Ser Ser Ser Tyr Thr Asp Leu Ser Arg Ser
        35                  40                  45
Ser Ser Pro Pro Ser Leu Leu Asp Gln Leu Gln Met Gly Cys Asp Gly
    50                  55                  60
Ala Ser Cys Gly Ser Leu Asn Met Glu Cys Arg Val Cys Gly Asp Lys
65                  70                  75                  80
Ala Ser Gly Phe His Tyr Gly Val His Ala Cys Glu Gly Cys Lys Gly
                85                  90                  95
Phe Phe Arg Arg Thr Ile Arg Met Lys Leu Glu Tyr Glu Lys Cys Glu
            100                 105                 110
Arg Ser Cys Lys Ile Gln Lys Lys Asn Arg Asn Lys Cys Gln Tyr Cys
        115                 120                 125
Arg Phe Gln Lys Cys Leu Ala Leu Gly Met Ser His Asn Ala Ile Arg
    130                 135                 140
Phe Gly Arg Met Pro Glu Ala Glu Lys Arg Lys Leu Val Ala Gly Leu
145                 150                 155                 160
Thr Ala Asn Glu Gly Ser Gln Tyr Asn Pro Gln Val Ala Asp Leu Lys
                165                 170                 175
Ala Phe Ser Lys His Ile Tyr Asn Ala Tyr Leu Lys Asn Phe Asn Met
            180                 185                 190
Thr Lys Lys Lys Ala Arg Ser Ile Leu Thr Gly Lys Ala Ser His Thr
        195                 200                 205
Ala Pro Phe Val Ile His Asp Ile Glu Thr Leu Trp Gln Ala Glu Lys
    210                 215                 220
Gly Leu Val Trp Lys Gln Leu Val Asn Gly Leu Pro Pro Tyr Lys Glu
225                 230                 235                 240
Ile Ser Val His Val Phe Tyr Arg Cys Gln Cys Thr Thr Val Glu Thr
                245                 250                 255
Val Arg Glu Leu Thr Glu Phe Ala Lys Ser Ile Pro Ser Phe Ser Ser
            260                 265                 270
Leu Phe Leu Asn Asp Gln Val Thr Leu Leu Lys Tyr Gly Val His Glu
        275                 280                 285
Ala Ile Phe Ala Met Leu Ala Ser Ile Val Asn Lys Asp Gly Leu Leu
    290                 295                 300
Val Ala Asn Gly Ser Gly Phe Val Thr Arg Glu Phe Leu Arg Ser Leu
305                 310                 315                 320
Arg Lys Pro Phe Ser Asp Ile Ile Glu Pro Lys Phe Glu Phe Ala Val
                325                 330                 335
Lys Phe Asn Ala Leu Glu Leu Asp Asp Ser Asp Leu Ala Leu Phe Ile
            340                 345                 350
Ala Ala Ile Ile Leu Cys Gly Asp Arg Pro Gly Leu Met Asn Val Pro
        355                 360                 365
Arg Val Glu Ala Ile Gln Asp Thr Ile Leu Arg Ala Leu Glu Phe His
    370                 375                 380
Leu Gln Ala Asn His Pro Asp Ala Gln Tyr Leu Phe Pro Lys Leu Leu
385                 390                 395                 400
Gln Lys Met Ala Asp Leu Arg Gln Leu Val Thr Glu His Ala Gln Met
                405                 410                 415
```

Met Gln Arg Ile Lys Lys Thr Glu Thr Glu Thr Ser Leu His Pro Leu
420 425 430

Leu Gln Glu Ile Tyr Lys Asp Met Tyr
435 440

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3301 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GAATTCTGCG GAGCCTGCGG GACGGCGGCG GGTTGGCCCG TAGGCAGCCG GGACAGTGTT 60
GTACAGTGTT TTGGGCATGC ACGTGATACT CACACAGTGG CTTCTGCTCA CCAACAGATG 120
AAGACAGATG CACCAACGAG GGTCTGGAAT GGTCTGGAGT GGTCTGGAAA GCAGGGTCAG 180
ATACCCCTGG AAAACTGAAG CCCGTGGAGC AATGATCTCT ACAGGACTGC TTCAAGGCTG 240
ATGGGAACCA CCCTGTAGAG GTCCATCTGC GTTCAGACCC AGACGATGCC AGAGCTATGA 300
CTGGGCCTGC AGGTGTGGCG CCGAGGGGAG ATCAGCCATG GAGCAGCCAC AGGAGGAAGC 360
CCCTGAGGTC CGGGAAGAGG AGGAGAAAGA GGAAGTGGCA GAGGCAGAAG GAGCCCCAGA 420
GCTCAATGGG GGACCACAGC ATGCACTTCC TTCCAGCAGC TACACAGACC TCTCCCGGAG 480
CTCCTCGCCA CCCTCACTGC TGGACCAACT GCAGATGGGC TGTGACGGGG CCTCATGCGG 540
CAGCCTCAAC ATGGAGTGCC GGGTGTGCGG GGACAAGGCA TCGGGCTTCC ACTACGGTGT 600
TCATGCATGT GAGGGGTGCA AGGGCTTCTT CCGTCGTACG ATCCGCATGA AGCTGGAGTA 660
CGAGAAGTGT GAGCGCAGCT GCAAGATTCA GAAGAAGAAC CGCAACAAGT GCCAGTACTG 720
CCGCTTCCAG AAGTGCCTGG CACTGGGCAT GTCACACAAC GCTATCCGTT TTGGTCGGAT 780
GCCGGAGGCT GAGAAGAGGA AGCTGGTGGC AGGGCTGACT GCAAACGAGG GGAGCCAGTA 840
CAACCCACAG GTGGCCGACC TGAAGGCCTT CTCCAAGCAC ATCTACAATG CCTACCTGAA 900
AAACTTCAAC ATGACCAAAA AGAAGGCCCG CAGCATCCTC ACCGGCAAAG CCAGCCACAC 960
GGCGCCCTTT GTGATCCACG ACATCGAGAC ATTGTGGCAG GCAGAGAAGG GGCTGGTGTG 1020
GAAGCAGTTG GTGAATGGCC TGCCTCCCTA CAAGGAGATC AGCGTGCACG TCTTCTACCG 1080
CTGCCAGTGC ACCACAGTGG AGACCGTGCG GGAGCTCACT GAGTTCGCCA AGAGCATCCC 1140
CAGCTTCAGC AGCCTCTTCC TCAACGACCA GGTTACCCTT CTCAAGTATG GCGTGCACGA 1200
GGCCATCTTC GCCATGCTGG CCTCTATCGT CAACAAGGAC GGGCTGCTGG TAGCCAACGG 1260
CAGTGGCTTT GTCACCCGTG AGTTCCTGCG CAGCCTCCGC AAACCCTTCA GTGATATCAT 1320
TGAGCCTAAG TTTGAATTTG CTGTCAAGTT CAACGCCCTG GAACTTGATG ACAGTGACCT 1380
GGCCCTATTC ATTGCGGCCA TCATTCTGTG TGGAGACCGG CCAGGCCTCA TGAACGTTCC 1440
ACGGGTGGAG GCTATCCAGG ACACCATCCT GCGTGCCCTC GAATTCCACC TGCAGGCCAA 1500
CCACCCTGAT GCCCAGTACC TCTTCCCCAA GCTGCTGCAG AAGATGGCTG ACCTGCGGCA 1560
ACTGGTCACC GAGCACGCCC AGATGATGCA GCGGATCAAG AAGACCGAAA CCGAGACCTC 1620
GCTGCACCCT CTGCTCCAGG AGATCTACAA GGACATGTAC TAACGGCGGC ACCCAGGCCT 1680
CCCTGCAGAC TCCAATGGGG CCAGCACTGG AGGGGCCCAC CCACATGACT TTTCCATTGA 1740
CCAGCTCTCT TCCTGTCTTT GTTGTCTCCC TCTTTCTCAG TTCCTCTTTC TTTTCTAATT 1800
CCTGTTGCTC TGTTTCTTCC TTTCTGTAGG TTTCTCTCTT CCCTTCTCCC TTCTCCCTTG 1860
CCCTCCCTTT CTCTCTCCTA TCCCCACGTC TGTCCTCCTT TCTTATTCTG TGAGATGTTT 1920
TGTATTATTT CACCAGCAGC ATAGAACAGG ACCTCTGCTT TTGCACACCT TTTCCCCAGG 1980
AGCAGAAGAG AGTGGGCCTG CCCTCTGCCC CATCATTGCA CCTGCAGGCT TAGGTCCTCA 2040
CTTCTGTCTC CTGTCTTCAG AGCAAAAGAC TTGAGCCATC CAAAGAAACA CTAAGCTCTC 2100
TGGGCCTGGG TTCCAGGGAA GGCTAAGCAT GGCCTGGACT GACTGCAGCC CCCTATAGTC 2160
ATGGGGTCCC TGCTGCAAAG GACAGTGGCA GACCCCGGCA GTAGAGCCGA GATGCCTCCC 2220
CAAGACTGTC ATTGCCCCTC CGATCGTGAG GCCACCCACT GACCCAATGA TCCTCTCCAG 2280
CAGCACACCT CAGCCCCACT GACACCCAGT GTCCTTCCAT CTTCACACTG GTTTGCCAGG 2340

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CCAATGTTGC TGATGGCCCC TCCAGCACAC ACACATAAGC ACTGAAATCA CTTTACCTGC    2400
AGGCACCATG CACCTCCCTT CCCTCCCTGA GGCAGGTGAG AACCCAGAGA GAGGGGCCTG    2460
CAGGTGAGCA GGCAGGGCTG GGCCAGGTCT CCGGGGAGGC AGGGGTCCTG CAGGTCCTGG    2520
TGGGTCAGCC CAGCACCTCG CCCAGTGGGA GCTTCCCGGG ATAAACTGAG CCTGTTCATT    2580
CTGATGTCCA TTTGTCCCAA TAGCTCTACT GCCCTCCCCT TCCCCTTTAC TCAGCCCAGC    2640
TGGCCACCTA GAAGTCTCCC TGCACAGCCT CTAGTGTCCG GGGACCTTGT GGGACCAGTC    2700
CCACACCGCT GGTCCCTGCC CTCCCCTGCT CCCAGGTTGA GGTGCGCTCA CCTCAGAGCA    2760
GGGCCAAAGC ACAGCTGGGC ATGCCATGTC TGAGCGGCGC AGAGCCCTCC AGGCCTGCAG    2820
GGGCAAGGGG CTGGCTGGAG TCTCAGAGCA CAGAGGTAGG AGAACTGGGG TTCAAGCCCA    2880
GGCTTCCTGG GTCCTGCCTG GTCCTCCCTC CCAAGGAGCC ATTCTATGTG ACTCTGGGTG    2940
GAAGTGCCCA GCCCCTGCCT GACGGNNNNN NNGATCACTC TCTGCTGGCA GGATTCTTCC    3000
CGCTCCCCAC CTACCCAGCT GATGGGGGTT GGGGTGCTTC TTTCAGCCAA GGCTATGAAG    3060
GGACAGCTGC TGGGACCCAC CTCCCCCCTT CCCCGGCCAC ATGCCGCGTC CCTGCCCCCA    3120
CCCGGGTCTG GTGCTGAGGA TACAGCTCTT CTCAGTGTCT GAACAATCTC CAAAATTGAA    3180
ATGTATATTT TTGCTAGGAG CCCCAGCTTC CTGTGTTTTT AATATAAATA GTGTACACAG    3240
ACTGACGAAA CTTTAAATAA ATGGGAATTA AATATTTAAA AAAAAAAGCG GCCGCGAATT    3300
C                                                                    3301
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
ACTCGGATCC AAGCCATGGC TGAGAACTTG CTGGACGG    38
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
CACAAAGCTT AGGCCATGTT AGCACTGTTC GG    32
```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CTCAGTCGAC TTATTGAATT CCACTAGCTG GAGATCC 37

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/21049

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07K 14/435, 14/705; C09K 11/06; G01N 33/53, 33/566
US CL :252/301.16, 301.36, 301.4R; 435/7.8; 530/350, 358
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 252/ 301.16, 301.32, 301.4R; 435/7.8; 530/350, 358

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P	ZHOU et al. Nuclear receptors have distinct affinities for coactivators: characterization by fluorescence resonance energy transfer. Molecular Endocrinology. October 1998. Vol. 12, No. 10, pages 1594-1604, especially page 1596 and figures 1-4.	1-27

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 DECEMBER 1998	21 JAN 1999

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	D. Lawrence Tor MICHAEL D. PAK
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS, STN, BIOSCIENCE, BIOSIS, CAPLUS, SCISEARCH

search terms: nuclear receptor, steroid receptor, retinoic acid receptor, co-activator, fret